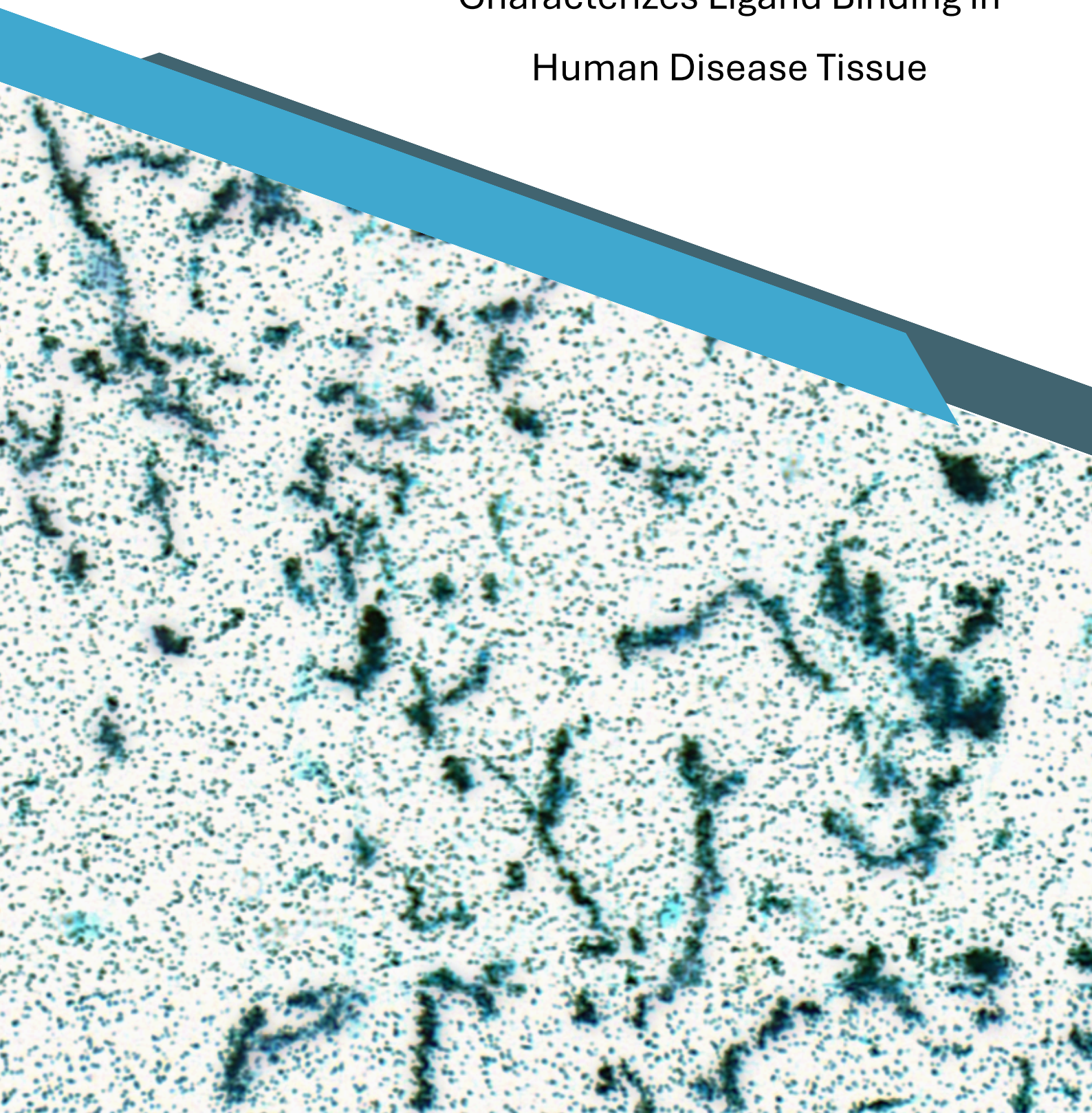
Seeing the Bond

How Receptor Autoradiography Characterizes Ligand Binding in Human Disease Tissue



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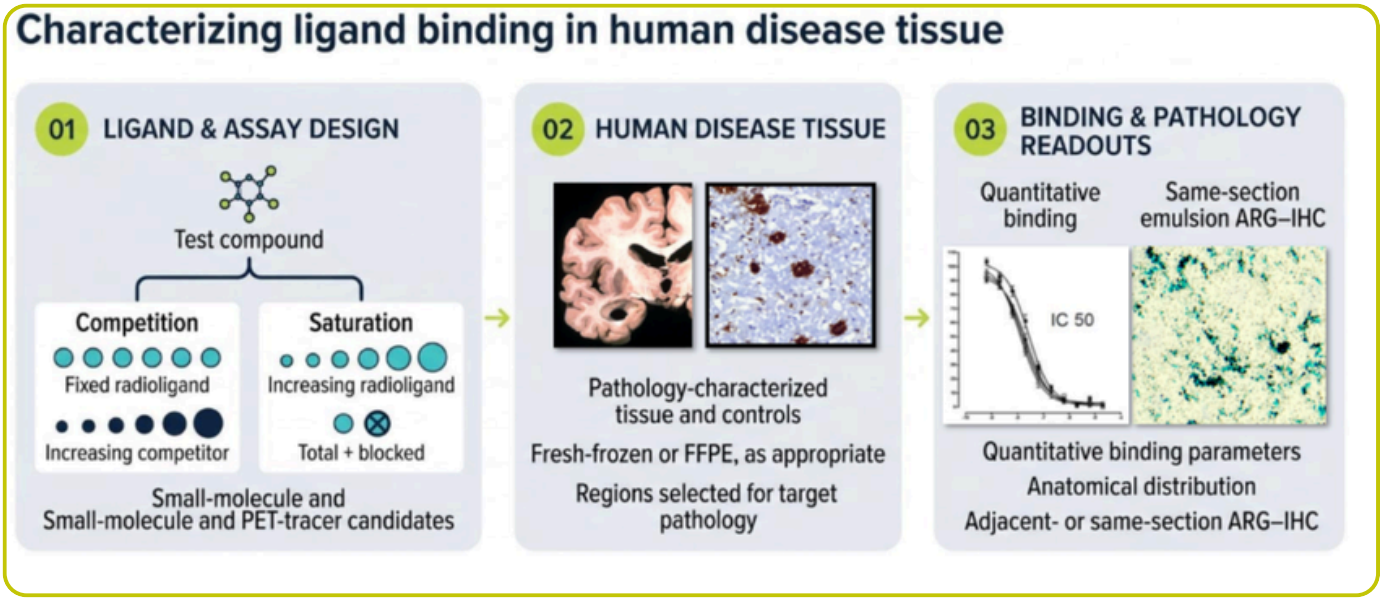
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Seeing the Bond

How Receptor Autoradiography Characterizes Ligand Binding in Human Disease Tissue

In central nervous system (CNS) drug discovery, demonstrating that a small molecule, PET tracer, or biologic binds its intended target is an important first step. However, binding data alone cannot establish whether the compound recognizes the intended pathology in human tissue. In neurodegenerative disease, where several protein aggregates and other pathological features can occur within the same region, understanding the precise location of a binding signal is essential to interpreting its significance, alongside meeting the affinity and selectivity discretion the program's pharmacology requires. Receptor autoradiography (ARG) addresses the first part of that question by measuring where a radiolabelled ligand binds in human disease tissue sections, how much it binds, and how that binding changes when unlabelled competitors are present. Displacement experiments characterize the affinity and pharmacological specificity of a small-molecule drug or PET-tracer candidate, and saturation experiments characterize the radioligand's affinity and available binding capacity. ARG is an in vitro method. It shows how a ligand behaves in tissue sections under defined conditions. It does not measure systemic exposure or blood-brain barrier penetration, and it cannot by itself show that a dosed compound occupies its target in vivo.



This article explains how we build the assay, where the radioligand and the tissue come from, why co-registering binding signal with target pathology makes the data interpretable, and what the resulting evidence can and cannot support. The technical detail stands on its own, and no prior familiarity with Offspring Biosciences' wider service architecture is needed.

For readers who know that context: ARG is one of five techniques in our Tissue Insights™ service Module 3, our offering for demonstrating target engagement and mechanism-of-action evidence in human disease tissue. Within Module 3, ARG supplies the pharmacological binding foundation, meaning the quantitative and anatomical profile of where a ligand binds and how strongly. It maps to Pfizer's Pillar 2 (binding) and supports AstraZeneca's "Right Target / Right Tissue" criteria for successful drug development. Molecular-contact evidence from isPLA and engagement evidence after dosing from in vivo immunodecoration build on that foundation. The companion Module 3 white paper gives the strategic rationale, but this article does not depend on it and can be read as a stand alone.

Displacement and Saturation: How the Assay Is Built

A single binding measurement, i.e. how much radiolabelled compound adheres to a tissue section, is not evidence of the specificity of its binding properties. Sticky, nonspecific binding can look identical to receptor binding in a raw image. This is addressed by an initial displacement study.

Tissue sections are incubated with a fixed concentration of the radiolabelled compound. Parallel sections also receive both the radioligand and an unlabelled reference or competitor compound at increasing concentrations. As the competitor concentration rises, it displaces radioligand from displaceable binding sites. We capture the resulting loss of signal on a phosphor-imaging system that allow us to quantify the IC50, the competitor concentration that halves specific binding under the stated conditions. A standard displacement series uses 10 competitor concentrations spanning five orders of magnitude, run in duplicate, with the radioligand at roughly half its Kd. We report an estimated inhibition constant (Ki) only when a one-site competitive equilibrium model is justified and the radioligand's Kd and free concentration are known under matching conditions.

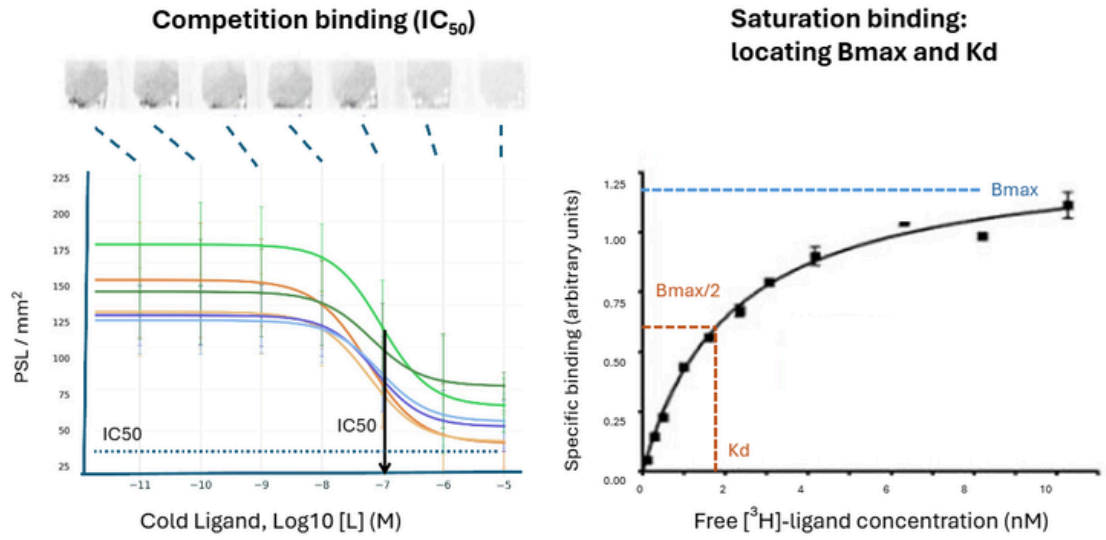
$$K_i = \frac{IC_{50}}{1 + [L]/K_{d,L}}$$

When these assumptions are uncertain, we report the IC50 and describe any Ki as an estimate.

Two further measurements define the components of the signal. A single large excess of unlabelled ligand defines the nonspecific component, and specific binding is the difference between total and nonspecific signal in matched sections. In this format "specific" means displaceable by the chosen competitor. It supports pharmacological specificity relative to that competitor and does not identify the molecular target by itself. Broader selectivity claims need several competitors, control and alternative-pathology tissue, and orthogonal evidence.

Saturation Experiments

To obtain the radioligand's apparent Kd and the available binding capacity (Bmax), we run a saturation series. Total and nonspecific binding are measured across a range of radioligand concentrations, and specific binding is fitted against free radioligand concentration in a justified model. A standard series uses 8–10 concentrations spanning roughly 0.1 to 10 times the Kd, in duplicate. During assay development we verify that binding has reached equilibrium with a time course, typically 5 to 120 minutes and extended where needed. We compare one-site and two-site fits using AICc, residuals, and parameter confidence, and report a one-site model unless a second site is reliably resolved. For fibrillar aggregates and other heterogeneous pathological deposits, we describe Bmax as available binding capacity under the assay conditions, as it need not equal total target protein.



Displacement and saturation binding: estimating IC_{50}, Kd and Bmax. ***Left:*** *Competition binding at a fixed radioligand concentration. Increasing concentrations of an unlabelled competitor reduce radioligand binding, illustrated by the autoradiographic images above the concentration–response curves. IC_{50} is the competitor concentration producing a 50% reduction in specific binding under the assay conditions.* ***Right:*** *Saturation binding measured across increasing free radioligand concentrations. Fitting specific binding - total minus nonspecific binding - to a one-site equilibrium model estimates the apparent dissociation constant (Kd) and available binding capacity (Bmax). Kd corresponds to the free radioligand concentration at half-maximal specific binding.*

Radioligands and Isotopes

Our work is primarily with tritiated compounds, and in some projects we also work with fluorine-18. Most radioligands are synthesized for the project by Novandi Chemistry, our preferred radiochemistry partner, with whom we have a long-term collaboration. Some clients send radioligands from their own radiochemistry units, and we work with both routes. Radiochemical purity and molar activity are verified by the supplying chemistry group, either the client's or Novandi's, and we always require a certificate of analysis. We use freshly prepared radioligand, and a radioligand that has been stored is re-tested before use.

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Tissue and Study Format

Receptor Autoradiography (ARG) studies at Offspring are run almost exclusively on human tissue, typically provided either directly from clients or through their partnering tissue banks. There is also the option to acquire tissue from tissue banks with established collaborations with Offspring Biosciences upon client request, including Netherlands Brain Bank, or to work directly with our more limited in-house tissue supply. Regardless of source, each tissue and sample is characterized for quality, anatomy, and pathology prior to analysis. Quality control includes H&E staining for morphological preservation and our proprietary IHC-based QC assay, which predicts the tissue's usefulness for our IHC, ISH, and ARG analyses. In our experience, higher-quality tissue gives lower background binding in ARG and better-preserved epitopes. Associated donor metadata is recorded as far back as can be supplied by the tissue provider.

A typical displacement study uses sections from one pathologically well characterized human case, with up to 30 slides and displacement of one to three radioligands. Studies conducted at Offspring are often run on tissue microarrays with autopsies/biopsies from up to 15 cases to more thoroughly account for variable pathology.

The study design selected for each project determines the scope of the conclusions. Multiple sections from one donor provide technical replication, while tissue microarrays allow comparisons across donors and variation in pathology. Sections from the same donor do not constitute independent biological replicates, and this distinction is made explicit in our reports.

The displacement framework can be run on fresh-frozen or FFPE-processed sections, depending on the target and the assay chemistry. Fresh-frozen cryosections are the usual preparation for receptor autoradiography, because they avoid formalin cross-linking and paraffin processing. FFPE is feasible for selected targets, but suitability must be shown for each ligand and target. A direct comparison with a p-tau PET tracer found that the better tissue format depended on the disease studied. Quantitative values from the two formats should not be pooled without a bridging analysis. For several ligands directed at proteinopathies, we have obtained comparable binding results from FFPE and snap-frozen tissue.

ARG was used in the development of AstraZeneca's amyloid-β PET ligand AZD4694 [Jureus, A., et al., J.Neurochem 2010 Aug;114(3):784-94.] with assistance and co-authorship by Offspring founders.

Assay formats are designed based on the custom needs of each project and our in-house subject matter expertise here at Offspring. One limitation we have observed in FFPE tissue is increased nonspecific binding to white matter tracts, particularly when our tissue QC indicates lower tissue quality. The choice of tissue route is the first decision point that determines whether, and how, binding data can be co-registered with pathology.

Receptor autoradiography extends beyond this core design and beyond neuroscience. In the wider field it supports oncology work on somatostatin receptor density for peptide receptor radionuclide therapy, whole-body ADME and toxicology studies, and biodistribution studies of radiolabelled antibodies and bioconjugates. Our own practice concentrates on neurodegenerative disease targets and fibrillar aggregate proteins, reflecting our scientific focus and not a limit of the technique.

Why Binding Data Alone Is an Incomplete Answer

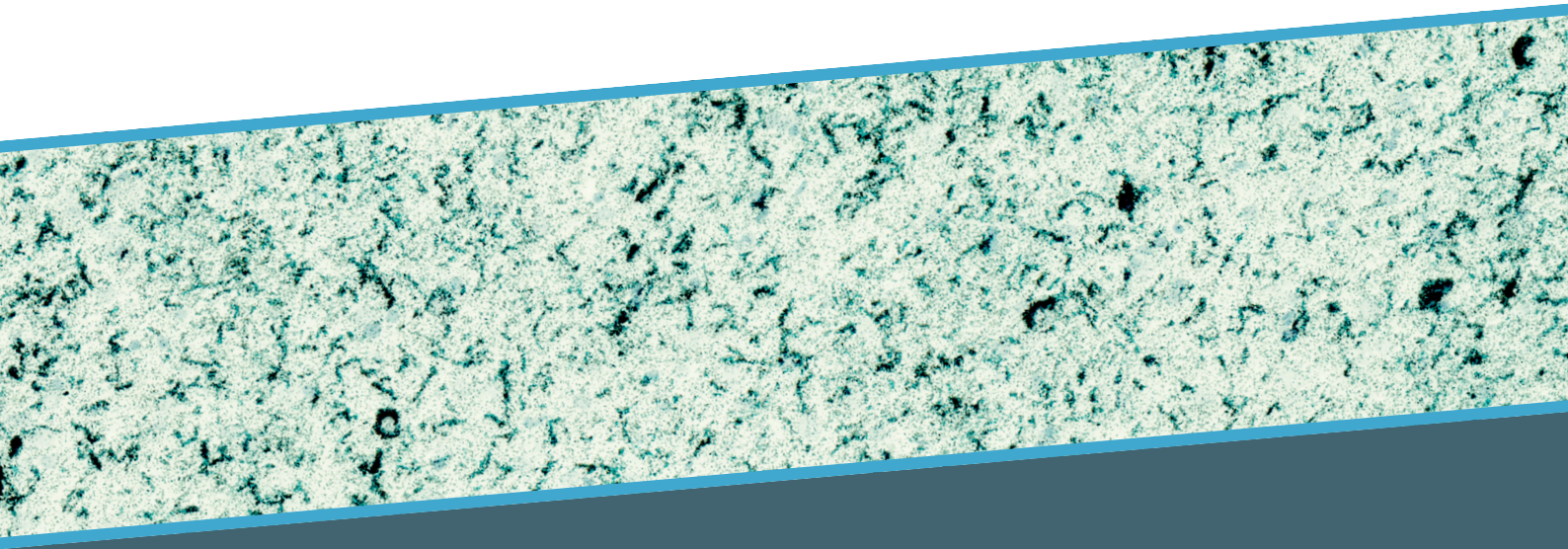
A displacement curve supports the conclusion that a compound binds in a displaceable, concentration-dependent way somewhere in the tissue section but fails to show co-localization of ligand binding relative to disease pathology and the target protein. For most translational programs that spatial relationship is the primary goal of the study. A binding signal anatomically dissociated from the pathological structures of interest (e.g. amyloid plaques, tau tangles, α-synuclein aggregates, receptors on tumour cells) raises exactly the question a governance committee or reviewer is trained to ask.

We use two co-registration approaches as routine parts of our ARG service and choose between them according to what the specificity question requires.

Consecutive-Section Co-Registration

At Offspring, we perform IHC for the relevant pathology marker on a section immediately adjacent to the one used for the ARG binding study, and align the two images digitally using shared anatomical landmarks, such as blood vessels, ventricles, other anatomical features, and pathological changes. This relates binding signal to the distribution of pathology at a regional, laminar, or lesion level, which is sufficient for many questions. We run it routinely wherever the question does not require cellular resolution on single sections.

The limitation is inherently structural as adjacent sections are never identical, so fine-scale correspondence is approximate, and cell-level correspondence cannot be assumed. We scale our conclusions to the achievable registration accuracy.



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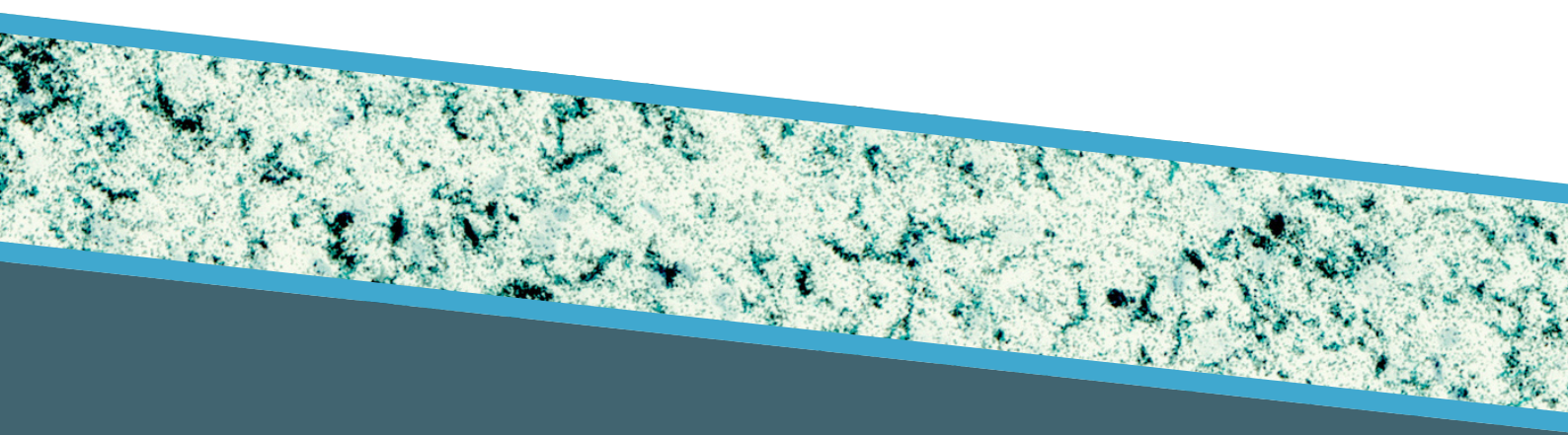
Dual Autoradiography–IHC on the Same Section

In one well-defined scenario, the structural limitation of adjacent sections becomes a real obstacle: ligands that may cross-react with structurally related target proteins that are physically intermixed within the same pathological structure. The clearest example is β-amyloid and tau, which frequently co-occur with tau-containing dystrophic neurites within and around Aβ plaques. On adjacent sections, a two-slide comparison cannot separate the two structures, so it cannot show which of them a cross-reactive ligand's signal is associated with.

For these cases we perform autoradiographic detection and IHC on the same physical section, using radiosensitive nuclear emulsion for the autoradiographic step. Emulsion records radioactive emission as silver grains over the stained tissue, which localizes signal at a much finer scale than phosphor imaging. Reading the grain pattern and the identity of the underlying structure from a single tissue plane removes the plane-mismatch problem, and published tau-tracer studies have used the same strategy in human brain tissue.

To decide whether binding coincides with a pathological structure, we use biomarkers of the pathological change and assess whether the binding signal localizes to the same cell or tissue structure as the biomarker. Antigen preservation is checked through our tissue proprietary QC testing assay run on adjacent sections.

Same-section analysis removes one source of uncertainty. Others remain: emission range and grain dispersion, antibody validity, lesion geometry, and protocol compatibility. This capability applies most consistently to fibrillar aggregate proteins such as α-synuclein, β-amyloid, and tau. Feasibility depends on whether the fixation and antigen-retrieval conditions required for IHC preserve the conformational binding epitope the radiolabelled compound recognizes, and the literature notes that IHC fixation can abolish or alter ligand binding. For programs with structurally distinct target classes, compatibility is established case by case.



From Visual Signal to Quantitative Data

The qualitative image, with dark regions where the compound has bound and faint or absent signal on competitor-blocked sections, is the first product of the assay. Its value increases when we convert it into quantitative parameters. Radiosensitive phosphor screens are exposed to the labelled sections and read on a calibrated Molecular Dynamics PhosphoImager system, referenced against standards of known radioactivity, using commercial tritium standards on plastic strips, and we analyse the images in MultiGauge software. Tissue is corrected for self-absorption, including differences between gray and white matter. The calibrated signal gives bound radioactivity in the tissue. Phosphor imaging offers high sensitivity and a broad linear range for regional work. The pharmacological parameters used come from the experimental design:

Parameter	Experimental Basis	How We Report It
IC50	Competition/displacement concentration–response series	Concentration giving 50% inhibition under stated conditions
Ki	Competition/displacement series, with radioligand concentration and an appropriate radioligand Kd, analysed using a competitive equilibrium model	Estimated equilibrium binding affinity of the competitor, conditional on the model assumptions
Kd	Equilibrium saturation series measuring total and nonspecific binding	Apparent equilibrium dissociation constant of the radioligand in the specified tissue preparation
Bmax	Equilibrium saturation series; quantitative calibration required for absolute binding-site density	Available binding capacity under assay conditions

Radiosensitive phosphor screens are exposed to the labelled sections together with commercial tritium standards on plastic strips, and the images are read on a calibrated Molecular Dynamics PhosphoImaging scanner and analysed in MultiGauge. Tritium's low emission energy means that tissue absorbs part of the signal, and more so in lipid-rich white matter than in gray matter. Signal is converted to tissue-equivalent radioactivity using tritium standards calibrated against tritiated tissue. The calibrated signal gives bound radioactivity per unit tissue. Imaging-plate resolution is typically on the scale of tens of micrometres, which supports regional and some microregional analysis.

The phosphor-based quantification described above belongs to autoradiography and is separate from our Visiopharm-based digital pathology pipeline (Pixels to P-values™), which Module 3 uses for IHC-based techniques such as multiplexed IHC. In same-section studies, the regional binding parameters come from phosphor imaging, and the emulsion image provides cellular-level localization of the binding signal relative to pathology.

These parameters describe drug–target interaction in tissue that retains its disease-relevant architecture. They are quantitative binding measurements and can inform the design of occupancy and PK/PD studies. Determining receptor occupancy and clinical dose requires exposure and occupancy data from dosed systems, which this in vitro design does not generate.

Autoradiography shows tau-associated binding of PET tracer OXD-2314 in human brain

Matrix: FFPE | Detector: phosphor screen, emulsion |
Readouts: total vs blocked binding, same-section AT8 | Targets: tau

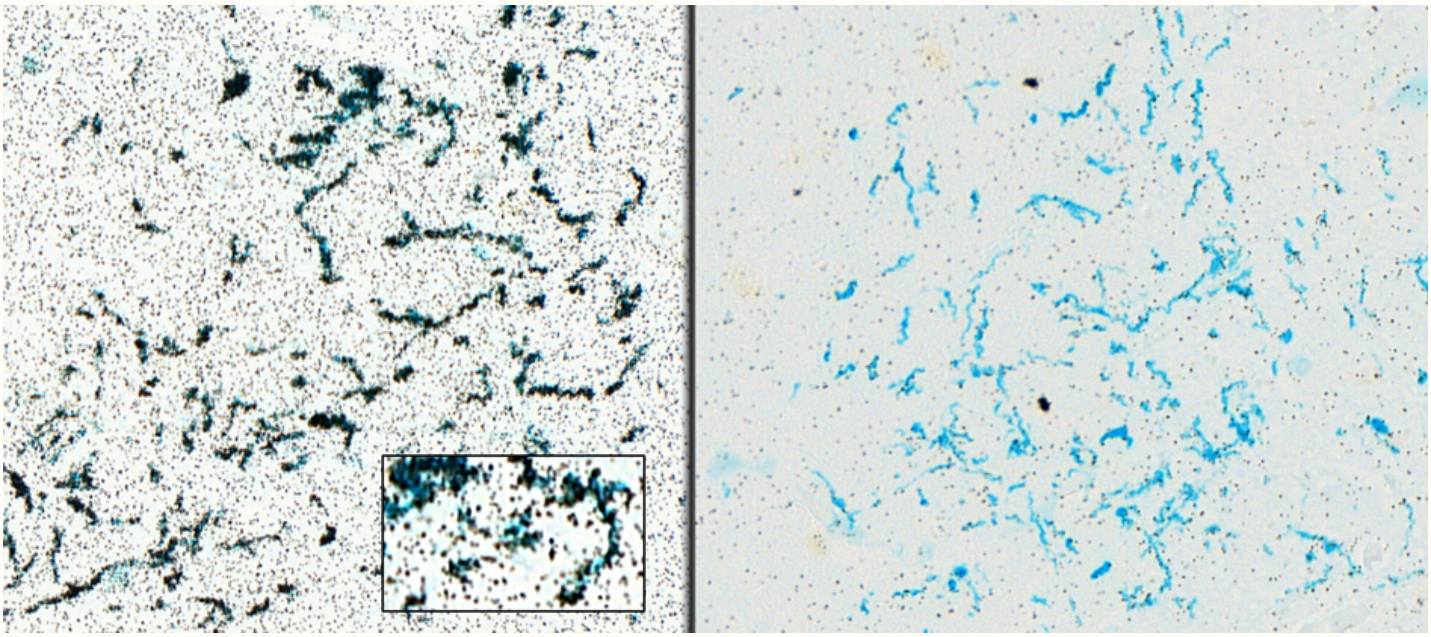
Client problem: [18F]OXD-2314 was developed as a PET tracer for tau aggregates in non-Alzheimer's tauopathies. Homogenate assays showed high affinity but cannot show where the ligand binds in intact tissue. The program needed independent evidence that [3H]OXD-2314 binds in a pattern consistent with tau pathology in human tissue, with displaceable signal and low binding in healthy control tissue.

Offspring solution: Offspring analysed an independent cohort of FFPE human brain sections from 2 PSP, 4 CBD, 1 AD and 1 healthy control case. Phosphor-screen autoradiography with 3 nM [3H]OXD-2314 mapped total binding, with excess unlabelled compound on adjacent sections defining non-specific binding, alongside AT8 immunohistochemistry for phospho-tau. In 2 PSP and 2 CBD cases, Offspring combined AT8 staining with emulsion-based, high-resolution autoradiography on the same section, so that radioligand silver grains could be viewed directly over phospho-tau pathology.

Outcome. Binding was displaceable with unlabelled compound and low in aged healthy control tissue. It was higher in AD hippocampus and in cortical PSP and CBD tissue. In the same-section overlay, silver grains aligned with phospho-tau-positive structures. These data were in agreement with, and extended on, results generated by a team of scientists at University of Toronto.

Reference: Lindberg A, et al.. Nature Communications 2024; 15:5109.

Same-section autoradiography–IHC demonstrates displaceable OXD-2314 binding associated with phospho-tau pathology



Same-section emulsion autoradiography and AT8 immunohistochemistry in human CBD tissue. (A) Total binding of [3H]OXD-2314, detected as black silver grains, superimposed on AT8-positive phospho-tau pathology (turquoise). The inset illustrates grain accumulation over stained pathological structures. (B) An adjacent section incubated with the same radioligand concentration in the presence of excess unlabelled OXD-2314 shows markedly reduced grain accumulation over AT8-positive structures. ARG and IHC were performed on the same physical section within each panel. Residual grains in the blocked preparation represent nonspecific signal and autoradiographic background. Tissue: FFPE CBD brain, Frontal cortex. No Aβ deposits were detected by IHC in the analysed region (data not shown).

Case Study: Redirecting α-synuclein PET ligand

Tissue autoradiography shows a candidate α-synuclein PET ligand binds 4R tau pathology more than α-synuclein

Matrix: FFPE I Detector: phosphor screen, emulsion I Readouts: aSYN IHC and emulsion based radioligand detection on consecutive tissue sections

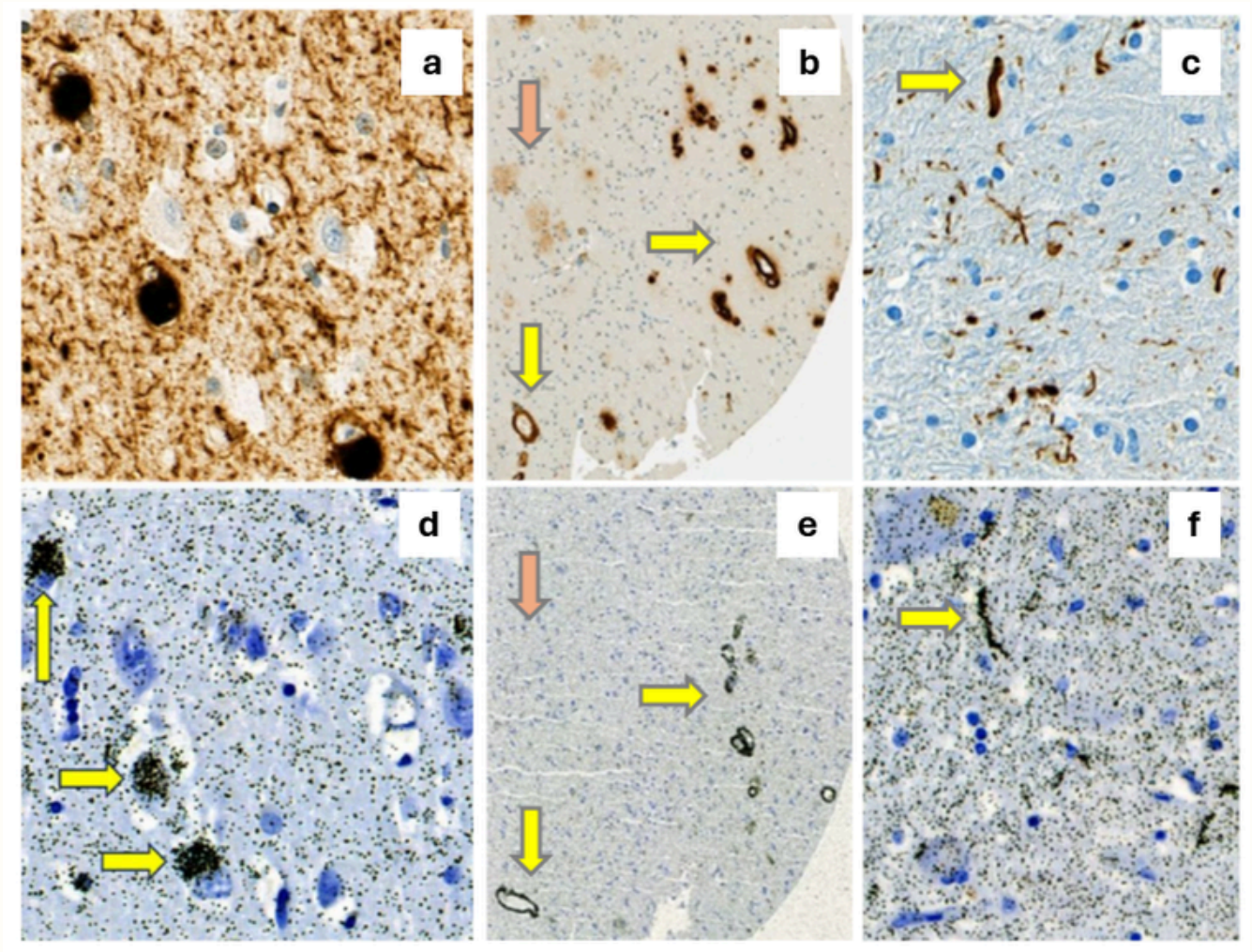
Client problem. A consortium of pharmaceutical companies and academic partners was developing a selective PET ligand for α-synuclein. In vitro screening of 78 compounds identified [3H]GMC-058, with a Ki of 22.5 nM on recombinant α-syn fibrils, with measured Ki values of 1320 nM (tau) and 1490 nM (Aβ). Analyses on recombinant protein, however, do not show whether a ligand binds native α-syn aggregates in disease tissue, or whether control tissue gives a clean background.

Offspring solution. Offspring supported the consortium by curating post-mortem brain samples from the Netherlands and UK Brain Banks and characterizing them for tissue quality and pathology. Sections from the tissue samples, some of which were first assembled into tissue microarrays for conservative and effective tissue processing, corresponding to PD, MSA, PSP, CBD, AD, CAA (diseases with various mixed pathologies of fibrillar α-syn, p-tau and/or Aβ deposits), as well as from control brain, were incubated with 25 nM [3H]GMC-058. Non-specific binding was defined on adjacent sections using excess unlabelled blocker. IHC for α-syn, p-tau and Aβ on adjacent sections was used to assess whether the radiosignal followed each pathology.

Outcome. [3H]GMC-058 had the lowest control-tissue binding of three candidates, but showed limited specific binding in PD and MSA tissue. Emulsion autoradiography placed some signal at α-syn inclusions, and also at dense Aβ deposits in CAA. However, ligand binding was strongest in PSP and CBD, where signal appeared to localize with p-tau inclusions. [3H]GMC-058 was therefore judged unsuitable as an α-syn PET candidate, with its binding to aggregated pTau profile warranting further evaluation.

This analysis contributed to a scientific study by Andrea Varrone et al.. Cells 2025, 14; 869. Dan Sunnemark, Offspring, was a coauthor in the study.

Human tissue analysis challenges the α-synuclein selectivity of GMC-058



Row label	LBD: α-synuclein	CAA: Aβ	PSP: phospho-tau
IHC	a	b	c
Emulsion ARG	d	e	f

Pathology-associated [3H]GMC-058 signal in human brain tissue. *Upper row: immunohistochemistry for α-synuclein in Lewy-body disease (a), Aβ in cerebral amyloid angiopathy (b), and phospho-tau in progressive supranuclear palsy (c). Lower row: high-resolution emulsion autoradiography with 25 nM [3H]GMC-058 on adjacent sections (d–f). Silver-grain accumulation occurs in regions corresponding to α-synuclein-positive inclusions, dense vascular Aβ deposits, and phospho-tau-positive structures. Yellow arrows indicate representative structures. Orange arrows indicate diffuse Aβ staining and the corresponding region without conspicuous grain accumulation in this example. These adjacent-section comparisons support qualitative pathology association rather than exact same-section co-localization. Panels are shown at different magnifications and are not intended for quantitative comparison of grain density.*

Where ARG Fits in the Module 3 Evidentiary Chain

ARG contributes one part of a coordinated system. It occupies a specific position in our five-technique Module 3 system, and understanding that position, including its boundaries, matters for interpreting what the data can claim.

Question	Technique Responsible
Does the candidate ligand bind its target in human disease tissue, with what affinity, pharmacological specificity, and anatomical distribution?	Receptor autoradiography (this article), including quantitative analysis of phosphoimaging data with MultiGauge
Is the drug in molecular contact with its target, at nanometre-scale resolution, within intact tissue?	In situ proximity ligation assay (isPLA)
Did the drug engage its target following systemic dosing, under real pharmacokinetic conditions?	In vivo immunodecoration
Did target engagement produce the intended downstream biological consequence?	Multiplexed IHC
Can the observed effect be expressed as a statistically robust output?	Quantitative image analysis (Pixels to P-values™)

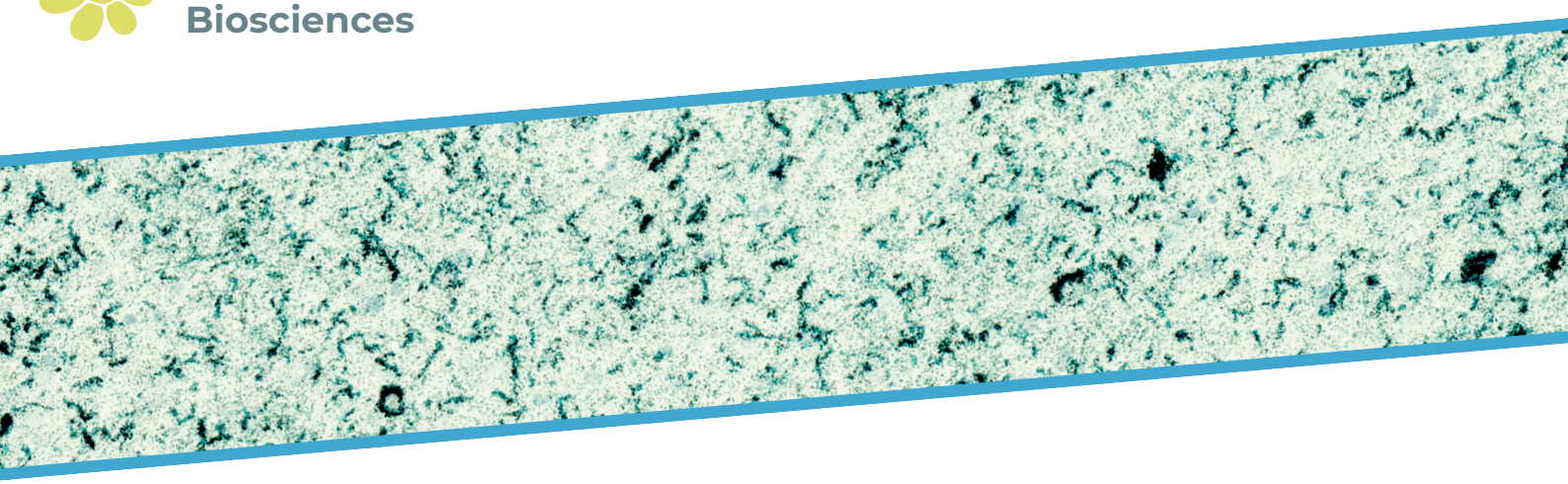
ARG establishes the regional, quantitative binding profile and its anatomical relationship to pathology. This is a different claim from the nanometre-scale contact evidence generated by isPLA. A well-characterized regional binding profile should not be read as proof of direct molecular engagement, and Module 3 treats these as separate questions answered by separate techniques. Programs that need both deploy ARG and isPLA together within the same tissue program.

Where This Evidence Is Used

An ARG study generates evidence that does specific work at several points in a program:

- **Mechanism documentation**. It provides pharmacological binding data in human disease tissue, which complements the animal-model data that mechanism-of-action documentation often relies on. Whether it contributes to a regulatory package depends on the context of use and the data package as a whole.
- **Design of occupancy and dosing studies.** Affinity and binding capacity in human tissue inform which compound concentrations and radioligands to use in later occupancy studies, which in turn feed PK/PD modeling.
- **PET tracer selection.** For tracer programs, binding and selectivity data in human tissue are often a prerequisite for tracer selection, since they show whether a radiolabelled compound has the target specificity and low off-target signal a viable imaging agent requires. Brain uptake, radiometabolites, kinetics, and in vivo quantifiability still need separate demonstration.

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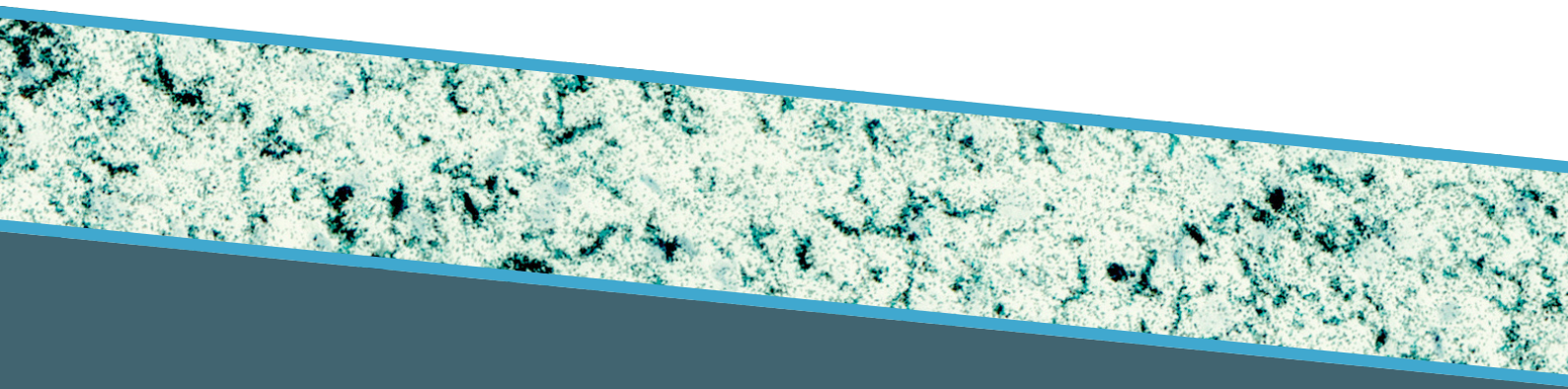
Limitations in ARG Data and Application

For a defensible interpretation, readers should note the following boundaries:

- **Exposure and BBB.** Section incubation does not model systemic exposure, BBB penetration, metabolism, or clearance.
- **Occupancy and dose**. In vitro binding does not determine in vivo occupancy or a clinical dose.
- **Specificity.** Displacement defines specificity relative to the competitor used.
- **Tissue state.** Post-mortem interval, disease stage, medication, storage, and processing can alter available binding.
- **Kinetics and models.** Kd, Ki, and Bmax interpretation depends on equilibrium and model assumptions, and washing can remove fast-dissociating binding preferentially.
- **Heterogeneous sites.** Aggregates may carry several binding-site classes that a one-site model would not capture.
- **Function.** Binding does not show downstream functional activity.

Analysis of Tissue from Dosed Animals

We do not offer ex vivo ARG study design or in vivo dosing. If a client provides tissue from animals treated in vivo with a radioligand, we can analyse selected organs by autoradiography. We are not set up for whole-body autoradiography. In these tissues, interpretation depends on the client's dosing and exposure design, and drug dissociation during tissue processing can bias occupancy estimates downward.



What We Deliver

- Displacement and saturation studies with tritiated radioligands in human disease tissue, on fresh-frozen or FFPE sections, with fluorine-18 ligands in some projects.
- Radioligands are typically provided by the client's own radiochemistry unit or via our collaborator, Novandi Chemistry.
- Tissue characterization for quality, anatomy, and pathology, and tissue sourcing from collaborating banks on request.
- Phosphor-imaging quantification against calibrated standards.
- Consecutive-section and same-section (emulsion) co-registration with IHC.
- Studies of up to about 30 slides (one to three radioligands) or tissue microarray studies with up to 15 cases.
- Autoradiographic analysis of selected organs from client-dosed animals, excluding whole-body autoradiography.
- Reports as short-format PowerPoint, with full written reports on request.

Contact Us

Speak directly with one of our senior translational scientists today. We will review your target biology and study parameters to determine the most defensible experimental configuration for your development pipeline, with no commercial obligation.

Receptor autoradiography is fundamentally about data defensibility. While a standard displacement curve can confirm that a compound has affinity under defined buffer conditions, it cannot tell a project team whether that binding tracks with the microscopic architecture of human disease. In translational neuroscience and complex proteinopathies, treating tissue as a uniform substrate leaves critical blind spots that surface later—usually when an internal governance committee or regulatory reviewer asks for spatial proof of specificity.

By pairing rigorous pharmacological design—10-point displacement curves, verified equilibrium conditions, and calibrated phosphor imaging—with anatomical and same-section emulsion co-registration, ARG anchors binding signals directly to the underlying pathology. It provides definitive, quantifiable evidence of where a ligand binds, how much available capacity exists, and whether structural cross-reactivity is a real concern. Recognizing what in vitro binding can prove—and transparently acknowledging what it cannot—ensures that when you take your candidate forward, your target engagement data stands up to peer interrogation.

To see how our modular framework addresses target validation, antibody selectivity, and translational de-risking across human disease cohorts, explore our full library of technical articles, case studies & white papers at

OffspringBiosciences.com