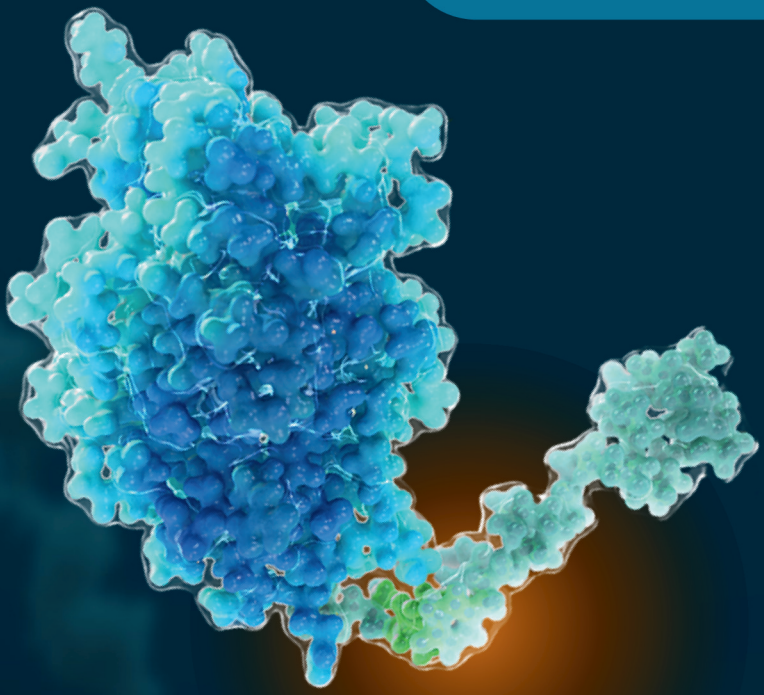
QVQ

QUALITY IN ANTIBODIES

Functionalizations of single-domain antibodies

Conjugation Services & Reagents



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QVQ
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Contents of this booklet

QVQ is your experienced partner for generating single-domain antibody panels

Customized project plan

Various sdAb libraries

Goal-oriented panning

Competitive & Cross-reactive binders

NGS & Analyses

Production & Purification

Affinities & Kinetics

Epitope binning

Engineering & Optimization

Full IP ownership

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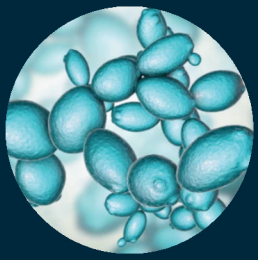
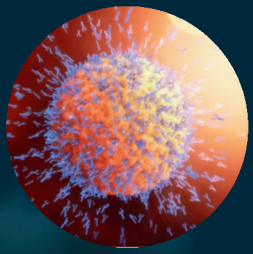
QUALITY IN ANTIBODIES

Production of sdAbs or fusion constructs from different hosts and at various scales

Bacterial strains

Yeast strains





Mammalian cells

Customized productions

- Tag of choice with or without extra cysteine for conjugation
- Genetic fusions: multivalents, Fc-domains, enzymes, etc
- Affinity-purified
- Up to 1 gram
- Quality control: integrity, purity, affinity, endotoxin level

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Directional conjugation of sdAbs

Proteins can be functionalized with many moieties

Protein conjugations have been around for almost a century but continue to evolve and become increasingly relevant. What started with radioactive labeling was later followed by fluorescent labeling and now extends to a myriad of functional groups including toxins, biotin, quantum dots, DNA, and many more. These modalities, and the introduction of multicolor measurements, have revolutionized the detection and quantification of proteins and protein interactions.(1)

Single-domain antibodies have several advantages

Today, functionalized antibodies also have important clinical applications in tumor imaging and treatment. Conventional antibodies are well advanced in these applications, but single domain antibodies (sdAbs) - also called VHHs or nanobodies - offer important advantages to this.(2) These antigen-binding fragments derived from camelid antibodies (Figure 1A) consist of only one domain and are therefore very stable. They are significantly smaller (~15 kDa) than full-size antibodies (~150 kDa), which allows for better tissue penetration and access to epitopes which might not be recognized by conventional antibodies.(3) The small size of sdAbs leads to rapid clearance from tissues, but if this is undesirable for a specific application, it can be addressed through various engineering approaches.(4) Also, sdAbs are generally more cost-effective and easy to reformat. Various sdAbs formats have already been clinically validated.(5–8)

Site-directed conjugation for sdAb labeling

sdAbs can easily be functionalized by attaching certain labels. The labeling of proteins is commonly performed using NHS esters which react with random lysines of the molecule. However, this method has some drawbacks: 1) Due to multiple lysines, this labeling leads to heterogeneity, which complicates reproducibility and stability; 2) In the case of fluorescence, a high degree of labeling can result in quenching. 3) Because lysines are often found in CDRs, crucial binding sites can be compromised and loss of functionality can occur.(9) For these reasons, site-specific protein labeling of sdAbs is often preferred.

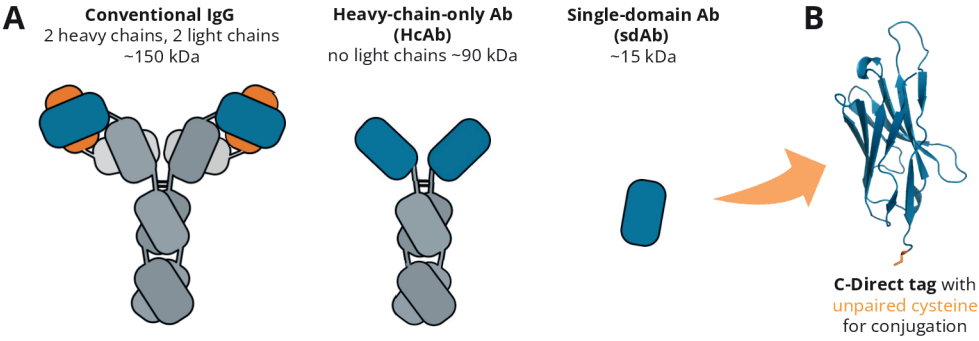


Figure 1. Structure of a sdAb. A) A conventional antibody (Ab) composed of two heavy chains and two light chains which need each other for stability (left). A heavy-chain-only antibody (HcAb) lacks light chains but remains stable (middle). A sdAb is the isolated binding domain of a heavy-chain-only Ab (right). B) Structure of a sdAb with C-Direct tag as predicted by AlphaFold2 (tag after cysteine is not shown).

Directional conjugation of sdAbs

C-direct tag for thiol-maleimide labeling

QVQ offers protein production of sdAb fused to our C-terminal C-direct tag. The C-direct tag contains an incorporated unpaired cysteine which can be labeled using a maleimide-thiol reaction. In this way, the label will be coupled on the opposite side of the epitope-binding paratope whereby binding affinity is maintained (Figure 2). QVQ has ample experience with the labeling of sdAbs or target proteins via genetic fusion, random labeling, and directional labeling via our C-direct tag. The resulting imaging probes have been validated in standard lab techniques such as ELISA, flow cytometry, and immunofluorescence. However, directionally functionalized sdAbs have shown their advantage in various, more advanced techniques. On the next pages are several examples where functionalized sdAbs developed by QVQ have been instrumental in advanced imaging techniques.

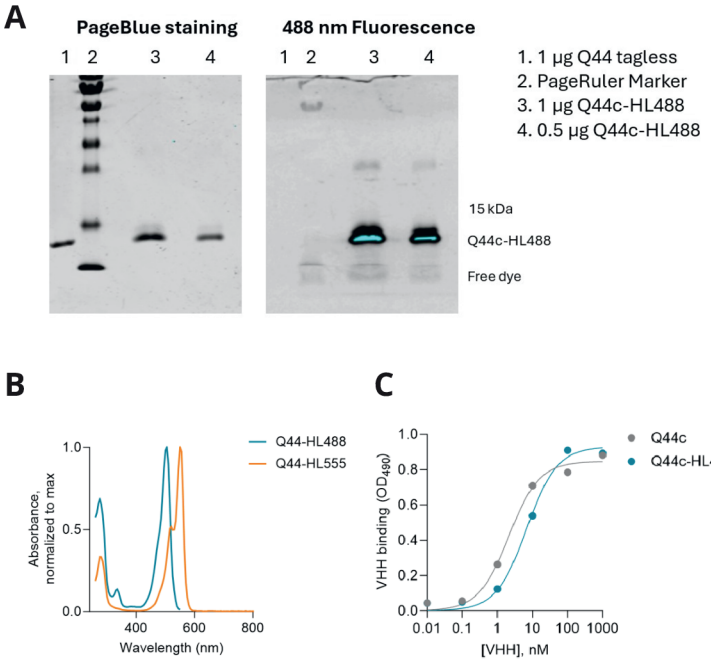


Figure 2. Quality Control of fluorescently labeled sdAb. A) SDS PAGE stained with PageBlue to assess protein integrity of fluorescently labeled sdAb Q44c (left) and scanned fluorescently to determine the amount of free dye (right). B) UV-Vis scan of fluorescently labeled sdAb Q44c to determine the degree of labeling (DOL). C) Binding affinity of conjugate determined by ELISA.

References:

1 Lakowicz (2006) doi:10.1007/978-0-387-46312-4.
2 Lameris et al. (2014) Crit Rev Oncol Hematol 92, 153–165.
3 Revets et al. (2005) Expert Opin Biol Ther 5, 111–124.
4 Holt et al. (2008) PEDS 21, 283–288.
5 Scully et al. (2019) N Engl J Med 380, 335–346.
6 Li et al. (2021) J Hematol Oncol 14.
7 Gondry et al. (2024) J Nucl Med 65, 178–184.
8 San-Miguel et al. (2023) N Engl J Med 389, 335–347.
9 Usama et al. (2021) Curr Opin Chem Biol 63, 38–45.

Functionalization Services

Our sdAbs can be conjugated in a site-directed manner via maleimide-thiol reactions to the following labels.

Fluorophores	Chelators	Proteins	Other
HiLyte488	DOTA	HRP	Biotin
HiLyte555	NOTA	Luciferases	Peptides
HiLyte647	DOTAGA	Fluorophores	Oligonucleotides
IRDye 680RD			
IRDye 800CW			

Alternatively, we offer **sdAb-DBCO** conjugates for the conjugation via Click Chemistry to **azide-modified labels** including fluorophores of different wavelength. In addition, we offer custom conjugations to your sdAb and label of interest.

Other functionalizations can be introduced by **genetic fusion**. QVQ offers the generation of multispecific and multivalent single-domain antibody constructs. In addition, we can fuse single-domain antibodies to different Fc-domains, peptides, or proteins. Other fusion proteins can be generated upon request.

Service Sheet

Generation of sdAb-fluorophore conjugates

Small-sized sdAb conjugates

Single-domain antibodies (sdAb, Figure 1) are small in size (~15 kDa) which gives them several advantages in imaging applications compared to full-length antibodies.[1] Because of the small distance between epitope and label, fluorescent sdAbs have a small linkage error making them suitable for the sensitive, direct detection of binding (e.g. flow cytometry) or protein-protein interaction (e.g. BRET).[2] Furthermore, sdAbs have good tissue penetration and rapid systemic clearance *in vivo* making them valuable tools for 3D and *in vivo* imaging.[3,4]

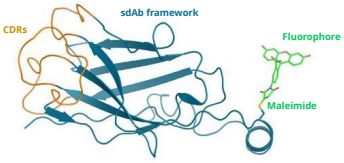


Figure 1. Structure model of sdAb-fluorophore conjugate. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to maleimide-modified fluorophore (green).

Organic dyes

Organic fluorophores are small chemical molecules that emit light after excitation. A diverse range of these dyes has been developed in the last decades, featuring various excitation and emission spectra covering the visible spectrum and extending into the near-infrared range (Figure 2).[5] Using small-molecule dyes keeps the overall size of the conjugate small retaining the benefits of sdAbs for detection and imaging applications.

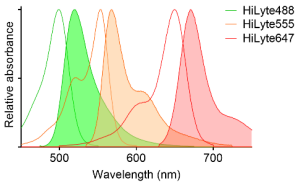


Figure 2. Spectra of HiLyte dyes. Excitation/ emission maxima: HiLyte488: 502/527 nm, HiLyte555: 552/569 nm, HiLyte647: 649/674 nm.

sdAb-fluorophore conjugation

QVQ generates sdAb-fluorophore conjugates via a Click Chemistry reaction between an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and a maleimide-modified dye. The resulting conjugate is checked for protein integrity, degree of labeling, and target binding (Figure 3).

sdAb conjugates are routinely generated with the fluorophores of the visible spectrum (ATTO- or HiLyteFluor dyes) , Figure 2) as well as the near-infrared dyes IRDye® 680RD and IRDye® 800CW. Other fluorophores can be conjugated on request.

Deliverables

- Fluorescently labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - UV-Vis spectrum
  - Percentage of free dye (SDS PAGE, fluorescent scan)
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity (ELISA)

Examples:

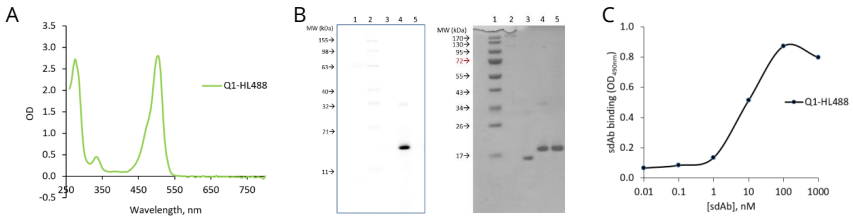


Figure 3. Example of quality control of generated sdAb-fluorophore conjugate Q1c-HL488. A) UV-Vis spectrum of sdAb-fluorophore conjugate Q1-HiLyteFluor488. B) SDS PAGE of 1) Marker, 2) Fluorescent Marker, 3) Reference sdAb (1 ug), 4) Q1c-488, and 5) unlabeled Q1c scanned for fluorescence (left) and after PageBlue staining (right). C) Binding of conjugate to recombinant HIV-encoded gp120 in ELISA. Bound sdAb was detected with rabbit-anti-VHH (QE19), followed by donkey-anti-rabbit-HRP and OPD as substrate.

References

1 Hamers-Casterman et al. (1993) Nature 363(6428), 446-448.
2 Van den Bor, Bergkamp et al (2023) Cell Rep. Methods 3(3), 100422.
3 Jeremiasse et al (2024) EMBO molecular medicine 16(7), 1495-1514.
4 Kijanka et al (2016) EJNMMI Res 6(1), 14.
5 Grimm and Lavis (2021) Nat. Methods 19, 149-158.

Service Sheet

Generation of sdAb-biotin conjugates

sdAb detection with biotin

Single-domain antibodies (sdAbs) are valuable detection agents due to their small size and specificity. Detection of antigen-bound sdAbs can be achieved via secondary antibodies or directly via conjugated labels.

One widely used label is biotin, a small, biological molecule that interacts with the much larger and tetrameric proteins avidin, streptavidin, and derivatives of those.[1] The interaction of avidin and biotin is one of the strongest non-covalent interactions known ($K_D \approx 10^{-15}$ M).[2] This makes the biotin-(strept)avidin complex a useful tool in various biochemical assays detecting proteins or protein-protein interactions.[3,4]

Biotinylated sdAbs (Figure 1) allow for capturing on (strept)avidin-coated surfaces and sensitive detection of sdAb binding and presence of antigens in e.g. ELISA and SPR.

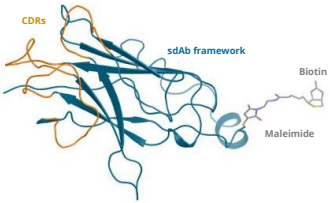


Figure 1. Structure model of sdAb-biotin conjugate. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to maleimide-biotin.

sdAb-biotin conjugation

sdAb-biotin conjugates are generated via a click reaction of an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and maleimide-modified biotin. The resulting conjugate is checked for protein integrity, degree of labeling by using 4'-hydroxyazobenzene-2-carboxylic acid (HABA), and target binding in ELISA, SPR, or flow cytometry (Figure 2).

Deliverables

- Biotin-labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity of the conjugate (ELISA, biotin detection)

Examples:

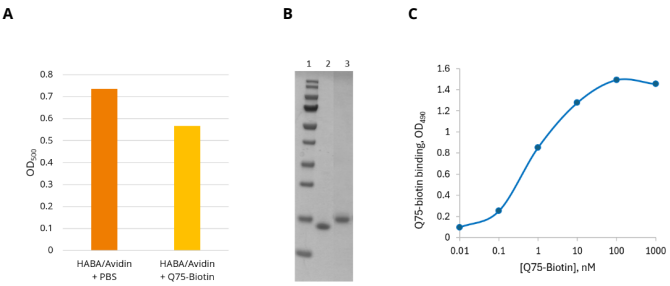


Figure 2. Example of quality control of conjugated sdAb-biotin. A) Results of HABA assay for the determination of DOL. B) SDS PAGE of 1) Marker, 2) Reference VHH (1 ug), and 3) Q75-biotin conjugate. C) sdAb-biotin binding to immobilized recombinant ITGB1 in ELISA detected with ExtrAvidin-HRP.

References:

1 Laitinen et al (2006) Cell. Mol. Life Sci. 63, 2992–3017.
2 Kuhn and Kollmann (2000) J. Am. Chem. Soc. 122, 3909-3916.
3 Ren, Han et al (2015) Chem. Commun. 51, 10403.
4 Dundas et al (2013) Appl Microbiol Biotechnol. 97(21), 9343-9353.

Service Sheet

Generation of sdAb-chelator conjugates

sdAbs as radiolabeled probes

Radiolabeled probes facilitate highly sensitive and quantitative molecular imaging through Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT).[1] To generate such probes, antibodies or antibody fragments are first labeled with chelators that can then be used to bind radioisotopes for imaging.[2] The small size of sdAbs allows for enhanced tissue penetration as compared to full-length antibodies and rapid clearance *in vivo*.[3,4]

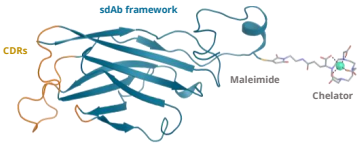


Figure 1. Structure model of sdAb-chelator. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to maleimide-DOTAGA with chelated ion (cyan).

sdAb-chelator conjugation

sdAb-chelators conjugates (Figure 1) are generated via a click reaction of an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and commercially available maleimide-modified chelators. The resulting conjugate is checked for protein integrity and target binding. sdAb-chelator conjugates can subsequently be used for radiolabeling and imaging or MRI.

QVQ offers sdAb conjugates with the maleimide-modified chelators DOTAGA, DOTA, and NOTA. Other custom conjugations can be requested.

Deliverables

- Conjugated sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity (ELISA)

References:

1 Wu and Olafsen (2008) Cancer J. 14(3), 191-197.
2 Morais and Ma (2018) Drug Discov. Today Technol. 30, 91-104.
3 Jeremiasse et al (2024) EMBO molecular medicine 16(7), 1495-1514.
4 van Ineveld et al., (2021) Nat Biotechnology, 39, 1239-1245

Service Sheet

Generation of sdAb-HRP conjugates

Horseradish Peroxidase

Horseradish peroxidase (HRP) is a 44 kDa protein derived from horseradish roots[1]. HRP is a metalloenzyme catalyzing the oxidation of organic substrates leading to the formation of chromogenic or fluorogenic products.[2,3] It is widely used as a reporter enzyme in various stainings and assays.[4] When combined with detection probes, such as antibodies, HRP can be employed for the sensitive detection of targets, for instance using ELISA.[4]

Detecting binding of primary probes in ELISA typically involves using secondary and even tertiary antibodies. Using directly labeled sdAb-HRP (Figure 1) allows for immediate detection shortening assay time drastically. Using sdAb-HRP also allows for a straightforward assessment of sdAb-sdAb competition (Figure 2).

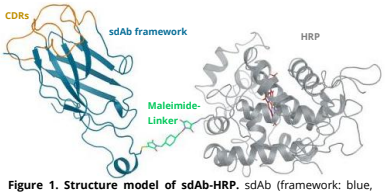


Figure 1. Structure model of sdAb-HRP. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to bifunctional linker (green) to HRP (grey).

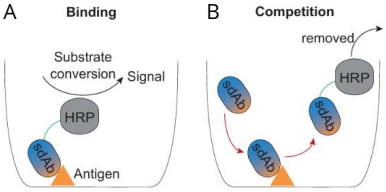


Figure 2. Illustration of sdAb-HRP in ELISA. sdAb-HRP binds to immobilized antigen. After washing away unbound protein, bound sdAb-HRP is quantified by colorimetric substrate conversion. This allows for detecting sdAb-HRP binding (A) and competition with unlabeled sdAbs (B).

sdAb-HRP conjugation

sdAb-HRP conjugates are generated by using a bifunctional linker targeting an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and a lysine of the HRP. The resulting conjugate is checked for protein integrity, degree of labeling, target binding, and HRP functionality (Figure 3).

Deliverables

- HRP-conjugated sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - Percentage of free HRP (SDS PAGE, PageBlue stained)
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity (ELISA)
  - Confirmation of HRP functionality (ELISA)

Examples:

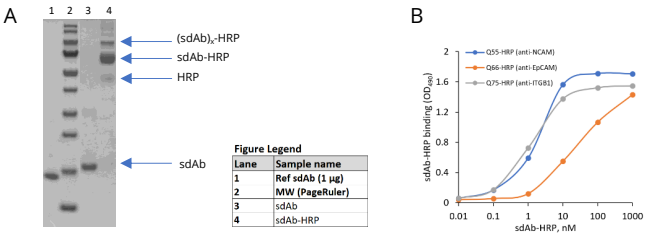


Figure Legend

Lane	Sample name
1	Ref sdAb (1 µg)
2	MW (PageRuler)
3	sdAb
4	sdAb-HRP

Example of quality control of generated sdAb-HRP conjugates. A) PageBlue stained SDS PAGE of sdAb-HRP conjugate. B) Binding of sdAb-HRP conjugates to immobilized recombinant antigens detected using OPD as substrate.

References:

1 Berglund et al. (2002) Nature 417(6887), 463–468.
2 Veitch (2004) Phytochem. 65(3), 249-259.
3 Meng et al. (2005) Anal. Biochem. 345(2), 227-236.
4 Krainer and Glieder (2015) Appl. Microbiol. Biotechnol 99, 1611-1625.

Service Sheet

Generation of sdAb-DBCO conjugates for click chemistry

sdAb conjugation

sdAbs can be easily functionalized by attaching labels, typically through NHS labeling of amines of lysines or site-directed thiol-maleimide coupling of cysteines. As lysines in sdAbs often contribute to their target binding, site-directed conjugation is the preferred approach.[1] For many labels, thiol-reactive maleimide-modified variants are commercially available. However, if these are not available, azide-modified labels can be a viable alternative as they react readily with dibenzocyclooctyne (DBCO).[2] In that case, sdAb-DBCO conjugates (Figure 1) are used as intermediate reagents.

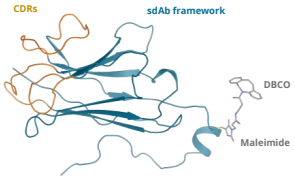


Figure 1. Structure model of sdAb-DBCO. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine to maleimide-DBCO.

DBCO

DBCO, also known as Dibenzoazacyclooctyne (DIBAC), is a molecule comprising two benzene rings and a cyclooctyne ring.[3] This 8-membered ring contains a C-C triple bond, which introduces significant strain and makes the molecule highly reactive.[3,4] In strain-promoted azide-alkyne cycloaddition (SPAAC) reactions, DBCO reacts readily with azides (Figure 2).[2,4] This reaction is a classic example of copper-free click chemistry and is widely used in bioconjugations covalently linking two molecules.[5,6]

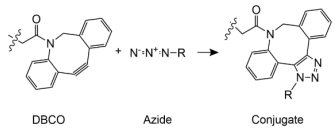


Figure 2. Reaction of DBCO and azide. DBCO (conjugated to sdAb) and azide (attached to label) undergo strain-promoted alkyne-azide cycloaddition (SPAAC) forming sdAb-label conjugate.

sdAb-DBCO conjugates allow the incorporation of azide-modified molecules, such as fluorophores, peptides, oligonucleotides, and more (Figure 3).

Deliverables

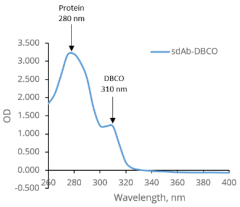
- DBCO-labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - UV-Vis spectrum
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity (ELISA)

sdAb-DBCO conjugation

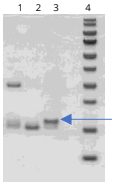
QVQ generates sdAb-DBCO conjugates via a copper-free click reaction of an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and maleimide-modified DBCO. The resulting conjugate is checked for protein integrity, degree of labeling, and target binding (Figure 3).

Examples:

A

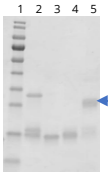


B



Lane	Sample
1	sdAb-c non-reduced
2	sdAb-c-DBCO
3	sdAb-c-DBCO-peptide
4	Marker

C



Lane	Sample
1	Marker
2	sdAb-c non-reduced
3	sdAb-c reduced
4	sdAb-c-DBCO
5	sdAb-c-oligonucleotide

D

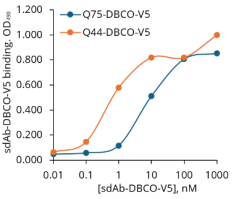


Figure 3. Example of quality control of generated sdAb-DBCO conjugates. A) UV-Vis spectrum for calculation of degree of labeling (DOL). B-C) SDS PAGE after conjugation of azide-modified peptide (B) or oligonucleotide (C) to sdAb-DBCO. D) sdAbc-DBCO-V5 tag conjugates binding to recombinant protein targets in ELISA. Bound V5-tagged sdAbs are detected using anti-V5-tag antibody.

References:

1 Massa et al (2014) Bioconjugate Chem. 25(5), 979-988.
2 Shi et al (2020) Angew. Chem. Int. 59(45), 19940-19944.
3 He and Cheng (2023) Molecules 28(9), 3715.
4 Albada et al (2021) Chem Rev 121, 7032-7058.
5 Wiener et al. (2020) Sci Rep 10, 1457.
6 Gong et al (2016) Bioconjugate Chem. 27(1), 217-225.

Service Sheet

Generation of sdAb-oligonucleotide conjugates

Oligonucleotides

Single-domain antibodies (sdAbs) are valuable tools for detecting proteins protein-protein interactions. Because of their small size, labeled sdAbs cause a small distance between target protein and label, which is referred to as small linkage error.[1]

Besides fluorophores, biotin, or enzymes, oligonucleotides have proven to be valuable labels in biophysical assays. In the form of single- or double-stranded pieces of DNA or RNA, oligonucleotides can be conjugated to various targeting probes.[2,3,4] In such biophysical assays, targeted oligonucleotides can undergo hybridization and ligation (in e.g. PLA) or extension (in e.g. PEA) and often amplification, making the assay highly sensitive. Subsequently, the probes can be detected using qPCR or labeled complementary oligonucleotides, either followed by direct quantification or (high-resolution) microscopy imaging.[3,4,5] In particular, the very small sdAb-oligo conjugates (Figure 1) allow for the sensitive detection of proteins and protein-protein interactions with a low linkage error.

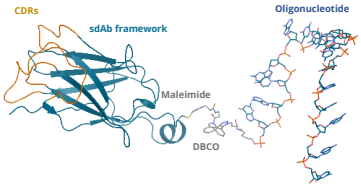


Figure 1. Structure model of sdAb-oligo. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine and DBCO to single-stranded oligonucleotide.

sdAb-oligonucleotide conjugation

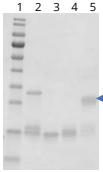
SdAb-oligonucleotide conjugates are generated by using maleimide-DBCO as bifunctional linker between an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and an azide-modified oligonucleotide. The resulting conjugate is checked for protein integrity, degree of labeling, and target binding (Figure 2). QVQ conjugates off-the-shelf sdAb products, as well as your sdAb of choice, to custom-ordered oligonucleotides.

Deliverables

- Oligonucleotide-labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity of the conjugate (ELISA detected with antisense-oligo)

Examples:

A



Lane	Sample
1	Marker
2	sdAb-c non-reduced
3	sdAb-c reduced
4	sdAb-c-DBCO
5	sdAb-c-oligonucleotide

B

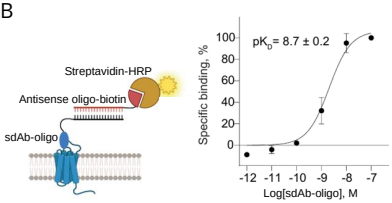


Figure 2. Example of quality control of generated sdAb-oligonucleotide conjugate. A) SDS PAGE after conjugation of azide-modified oligonucleotide to sdAb-DBCO. B) ELISA measuring binding of sdAb-oligonucleotides targeting CXCR4 to CXCR4-expressing HeLa cells. Binding of conjugate is detected by complementary DNA strand conjugated to biotin and streptavidin-HRP.

References:

1 Ries et al (2012) Nat. Methods 9, 582-584.
2 Gong et al. (2015) Bioconjugate Chem. 27(1), 217-225.
3 Al-Amim et al (2022) Anal. Chem. 94(28), 10054-10061.
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Service Sheet

Generation of sdAb-peptide conjugates

Peptides

Peptides can be valuable tools for functionalizing proteins of interest. For example, cell-penetrating or albumin-binding peptides can influence the uptake and in vivo biodistribution and final delivery of biologicals, such as antibodies.[1,2] In molecular research, short peptides frequently serve as epitope tags, enabling the detection, immobilization or purifications of proteins in various assays.[3,4]

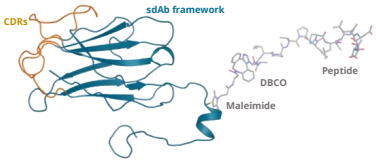


Figure 1. Structure model of sdAb-peptide. sdAb (framework: blue, CDRs: orange) conjugated via unpaired cysteine and DBCO to V5-tag peptide.

sdAb-peptide conjugation

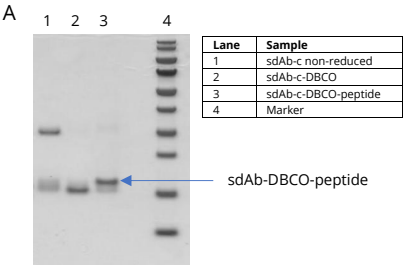
Single-domain antibody(sdAb)-peptide conjugates (Figure 1) are generated by using a maleimide-DBCO as bifunctional linker between an unpaired cysteine of the sdAb provided by e.g. our C-terminal C-direct tag and an azide-modified peptide. The resulting conjugate is checked for protein integrity, degree of labeling, target binding, and where applicable, functionality/recognizability of the peptide (Figure 2)

QVQ conjugates sdAbs to custom-ordered peptides.

Deliverables

- Peptide-labeled sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein parameters (MW, absorption/extinction coefficients)
  - Protein concentration, degree of labeling
  - Assessment of protein integrity (SDS PAGE, PageBlue stained)
  - Confirmation of target binding and apparent binding affinity (ELISA)

Examples:



Lane	Sample
1	sdAb-c non-reduced
2	sdAb-c-DBCO
3	sdAb-c-DBCO-peptide
4	Marker

Figure 2. Example quality control of generated sdAb-peptide conjugate. A) SDS PAGE gel of sdAb-DBCO conjugated to azide-V5 tag peptide. B) sdAb-V5 tag conjugates binding to recombinant protein targets in ELISA. Bound V5-tagged sdAbs are detected using anti-V5-tag antibody.

References:
1Dennis et al (2002) JBC 227(38), 35035-35043.
2 Guidotti et al (2017) TIPS 38(4), 406-424.
3 Marchetti et al (2023) FEBS Open Bio 13(12), 2239-2245.
4 Shevtsova et al (2006) EJN 23(8), 1961-1969.

Off-the-shelf reagents

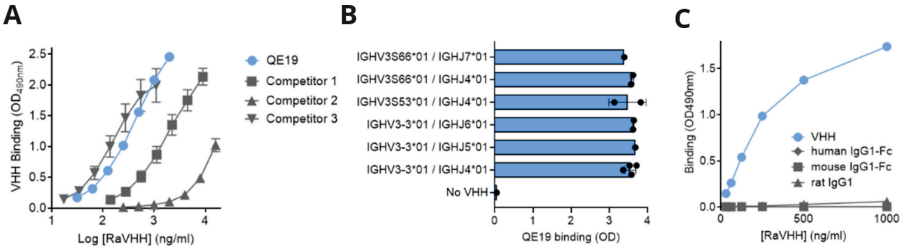
Target	sdAb
ACKR3	Q123, Q125, Q126c
B7-H3	Q92c
BMP2/4	Q36c
BMP4	Q35bc
CAIX	Q29c
Candidalysin	Q99c, Q100c
C5	Q101c, Q102c
CCR2	Q124c
CD63	Q111, Q112
CD80	Q98c
CD163	Q68c
CXCR4	Q84c, Q85c
DC-SIGN	Q51c, Q94c
EGFR	Q44c, Q86c
EpCAM	Q66c
Fc-domain	Q96c
Fibrinogen	Q113
Glycoprotein 1b α	Q114

Target	sdAb
Glycoprotein VI	Q115, Q116
HER2	Q17c
HIV gp120	Q1c
HIV gp41	Q8c, Q54c
HIV p24	Q76bc
IRDye800	Q93c
ITGB1	Q75c
cMet	Q22c
NCAM1	Q55c
PD-L2	Q90c
P-Selectin	Q117
SARS-CoV-2	Q103c, Q104c
TfR	Q52c
uPAR	Q88c
Vimentin	Q60c
VLA3	Q48c
vWF	Q118

Rabbit-anti-VHH

Building on our expertise of generating and characterizing single-domain antibodies, QVQ has developed a unique antibody against llama single-domain antibodies.

QE19 is a polyclonal protein A-purified rabbit antibody with little to no cross-reactivity to human, rat and mouse IgG1. QE19 recognizes all VHH germlines and is suitable for ELISA, immunofluorescence, and flow cytometry.



Detection of VHH by QE19. A) Detection of VHH by QE19 and three different competitor rabbit-anti-VHH. B) Detection of VHH from different germline families by QE19 (1:5000 dilution). C) Detection of Fc domains from human, murine or rat IgG1 by QE19. Bound antibodies were detected using donkey-anti-rabbit-HRP and OPD as substrate.

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Applications of sdAbs in fluorescent technologies and advanced imaging

The following pages showcase applications of QVQ's sdAbs in:

- Super-resolution imaging
- Bioluminescence Resonance Energy Transfer
- Photodynamic therapy
- Multicolor 3D tissue imaging
- In vivo imaging and image-guided surgery

Applications of sdAbs in fluorescent technologies and advanced imaging

Super-resolution imaging with fluorescent sdAbs

Super-resolution techniques allow imaging at the level of individual proteins and deliver sharp images to study structures and stoichiometry of targeted proteins, that otherwise cannot be resolved by standard imaging methods. sdAbs are very well suited for this since their limited size allows a very small distance between the label and the epitope on the antigen. This small linkage error creates fluorescent signals that are close to the true position of the antigen and results in a higher spatial resolution as compared to the use of conventional antibodies.(1) sdAbs can be conjugated to a enable their application in various super-resolution technologies such as Structured Illumination Microscopy (SIM) anddirect Stochastic Optical Reconstruction Microscopy (dSTORM) (Figure 1).

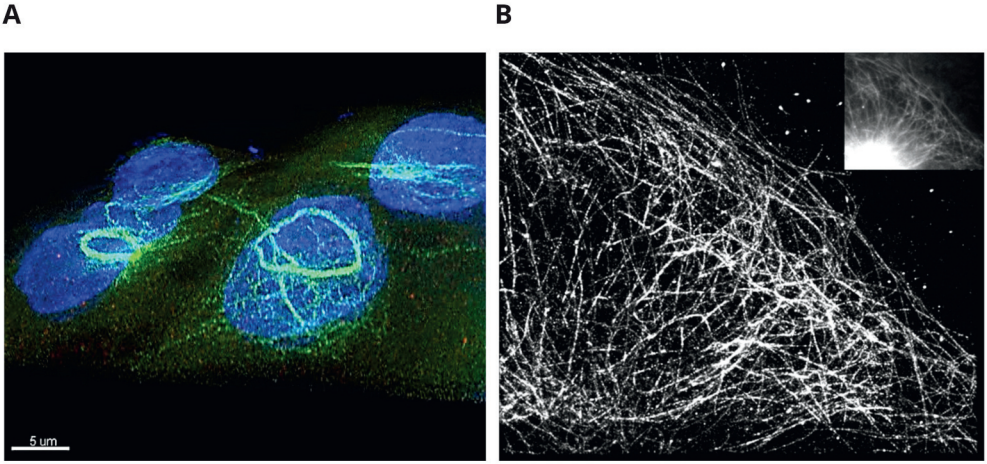

Figure 1. Fluorescently labeled sdAb used in super-resolution imaging. A) QVQ's anti-Vimentin sdAb, equipped with our C-terminal C-Direct tag (Q60c), was labeled with HiLyteFluor488 and subsequently used in SIM to reveal perinuclear vimentin rings in HeLa cells. B) Similarly, anti-tubulin sdAb (2), conjugated to the blinking HiLyteFluor647 showed tubulin fibers with high resolution using dSTORM.

Target engagement of fluorescent sdAbs by Bioluminescence Resonance Energy Transfer

Bioluminescence Resonance Energy Transfer (BRET) is a sensitive method to measure protein-protein interactions. This can be used to measure the binding of fluorescent sdAbs to luciferase-tagged targets (Figure 2A).(3) The energy transfer from luciferase to fluorophore only occurs when the probes are in very close proximity to each other and thus provides information on sdAb binding, including kinetics (Figures 2B and C).

This technique can also be used to study the competition of unlabeled compounds or to sense distinct target receptor conformations.(4,5) As the energy transfer from luciferase depends on the proximity of both probes, the reduced linkage error of sdAbs is also advantageous here. Therefore, fluorescent monovalent sdAbs give a higher and more robust BRET signal compared to fluorescent sdAb-Fc (Figure 2C).

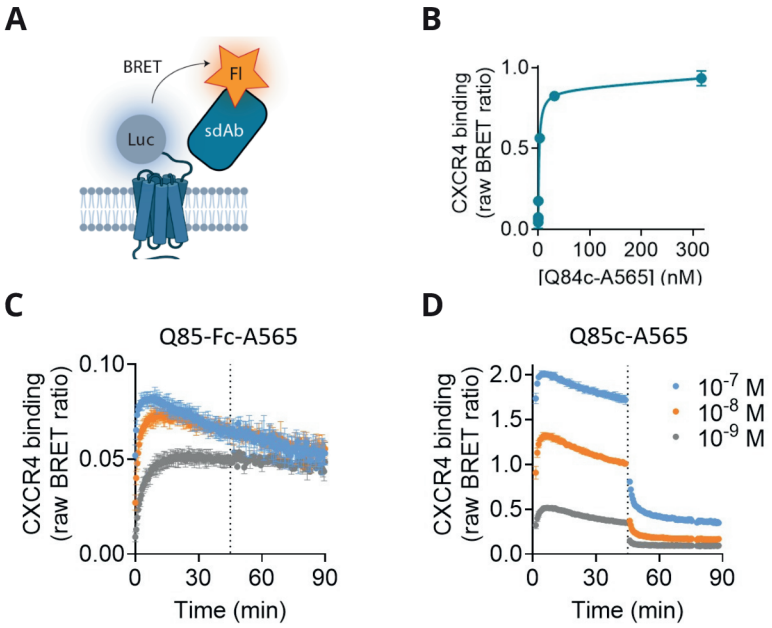

Figure 2. Study sdAb binding kinetics using BRET. A) Illustration of fluorescently labeled sdAb binding to CXCR4. B) Q84c-Atto565 binding to membrane extracts expressing luciferase (Luc) tagged CXCR4. Adapted from van der Bor et al.(4) C) Association and dissociation of Q85-Fc (left) and Q85 (right) binding to membrane extracts containing Luc-CXCR4. Adapted from van der Bor et al.(4)

Applications of sdAbs in fluorescent technologies and advanced imaging

Photodynamic therapy using photosensitizer-conjugated sdAbs

The fast clearance, good binding affinity, excellent tiddue distribution, and labeling with minimal risk of reduced binding make sdAbs highly suitable as targeting moieties of toxic substances for the treatment of e.g. solid cancers.

Immune activating or inhibiting sdAbs (6), toxic substances, or photosensitizing agents can be linked to the sdAb and thereby be delivered to its destination. For example, the CXCR4-targeting sdAb Q85c was conjugated to the very potent antimitotic agent monomethyl auristatin E to selectively kill CXCR4-overexpressing germ cell tumor cell lines.(6)

Also, light-inducible death of EGFR- and Met(HGFR)-overexpressing cells was shown by targeted photodynamic therapy using sdAbs conjugated to the photosensitizer IRDye700DX (Figure 3). (7,8)

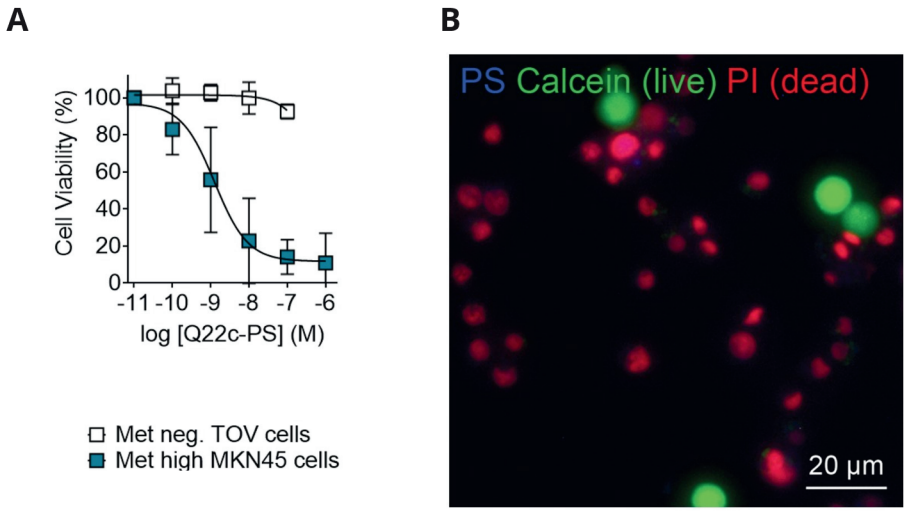

Figure 3. Targeting photodynamic therapy using sdAbs. A) Met (HGFR)-negative TOV112D or Met-overexpressing cells MKN45 cells were incubated with Met-specific sdAb Q22c, conjugated to the photosensitizer IRDye700DX (PS), and subsequently illuminated with near-infrared light to activate the photosensitizer. Cell viability was determined with resazurin. B) MKN45 cells treated as in A, after which cells were stained with CalceinAM (living cells), or propodium iodide (dead cells).(9)

Applications of sdAbs in fluorescent technologies and advanced imaging

Multicolor 3D tissue imaging with fluorescent sdAbs

3D imaging provides detailed information about the tissue's structure in all three dimensions, offering a more complete view compared to 2D imaging, which is limited to flat single-plane slices. This allows the study of biological structures and the spatial relationships between cells more extensively, improving analysis and understanding. The technique can also be combined with time-lapse imaging to observe tissue changes or movements over time, providing insight into developmental processes or disease progression.(10,11) With a size of 1/10 of a conventional antibody, sdAbs distribute more efficiently through tissues than conventional antibodies (Figure 4A).(10) Consequently, and in combination with their fast clearance, directly conjugated sdAbs are the ideal probes for imaging 3D spheroids, organoids and tissues.(10-12)

The potential of fluorescent sdAbs in 3D imaging is illustrated in Figure 6. QVQ's sdAb Q55 binding specifically to the extracellular domain of NCAM1 was conjugated to HiLyteFLuor555 and used to stain human fetal kidney (Figure 4B) (12). Q55c-HL555 was able to stain embryonic kidney tissues with excellent depth. Multispectral large-scale single-cell resolution 3D (mLSR-3D) imaging also showed whole kidney staining of NCAM with Q55c-HL555 (Figure 4C).(13) QVQ offers high-affinity sdAbs against multiple membrane-spanning proteins that can be used for 3D tissue imaging.

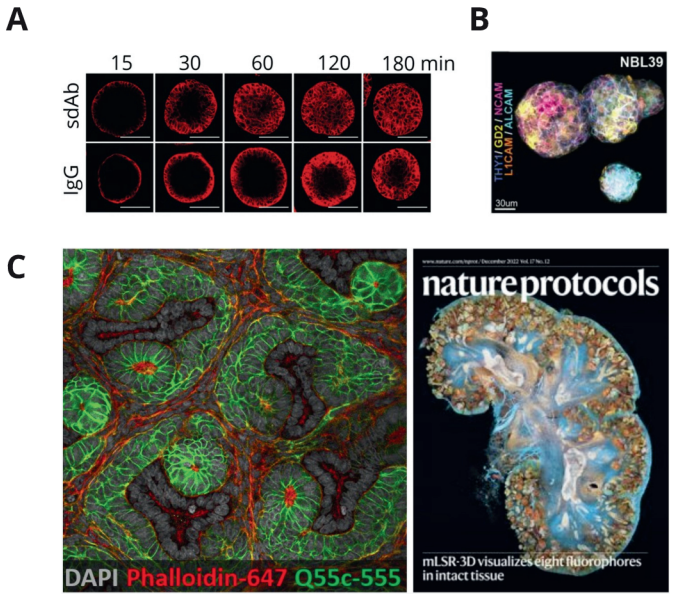

Figure 4. sdAbs allow deep tissue penetration and imaging. A)Epidermoid carcinoma A431 spheroids imaged with labeled, EGFR-specific sdAb or conventional antibody Cetuximab by confocal imaging microscopy. The scale bar is 100 µm. Adapted from (10). B) 3D multispectral imaging of tumor organoid. Anti-NCAM sdAb Q55c-HiLyteFluor555 is indicated in purple.(11) C) 3D imaging of human fetal kidney with Q55c-HiLyteFluor555 (green), actin-binding phalloidin-647 (red) and DAPI (grey).(12) D) Multispectral large-scale single-cell resolution 3D (mLSR-3D) imaging of a whole human fetal kidney. Q55c-HiLyteFluor555 is colored blue.(13)

Applications of sdAbs in fluorescent technologies and advanced imaging

In vivo imaging and image-guided surgery with near-infrared-fluorophore-labeled sdAbs

The relatively small size of sdAbs not only enables efficient penetration into solid tumors, but also results in fast clearance from the bloodstream (half-life of 1-2h).(14) These features combined with good target binding affinities create probes that are very useful to image solid tumors in vivo and generate good tumor-to-background signal ratios at much earlier time points than conventional antibodies. This was shown for two Single-Photon Emission Computed Tomography (SPECT) and near-infrared imaging.(15,16)

sdAb probes can also be applied in image-guided surgery. For example, QVQ's anti-Carbonic Anhydrase IX (CAIX) sdAb Q29c and anti-HER2 sdAb Q17c were used for dual-spectral intraoperative imaging of orthotopic breast cancer models with MCF10DCIS cells in mice.(16) This reached good tumor-to-background ratios already 2 hours after injection (Figure 5).

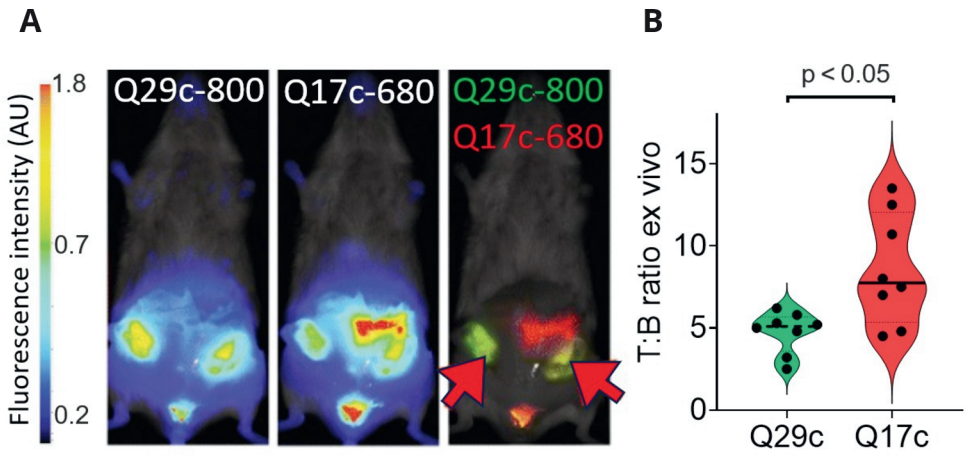

Figure 5. Dual-spectral intraoperative imaging using sdAbs. A) Dual-spectral, intraoperative, imaging of orthotopic MCF10DCIS breast cancer tumors in mice using anti-CAIX sdAb Q29c conjugated to IRDye800CW and anti-HER2 sdAb Q17c conjugated to IRDye680RD. B) Tumor-to-background fluorescent signal ratios for Q29c-800 and Q17c-680.(16)

References:

1 Ries et al. (2012) Nat. Methods 9, 582–584.
2 Mikhaylova et al. (2015). Nat. Commun. 6, 7933.
3 Dale et al. (2019), Front. Bioeng. Biotechnol. 7, 56.
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6 Wakileh et al. (2023) Exp. Hematol. Oncol. 12, 96.
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10 Beltrán Hernández et al. (2019) Mol. Imaging Biol. 21, 1079–1088.
11 Jeremiasse et al. (2024) EMBO Mol. Med. 16, 1495.
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16 Kijanka et al. (2016) EJNMMI Res 6, 1–13.

Contact us for the generation and/or functionalization of your sdAb.

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