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Visualization of vitamin A metabolism

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Chapter 1

General Introduction

Aldehyde dehydrogenases

The aldehyde dehydrogenases (ALDHs) belong to a family of 19 oxidoreductases based on sequence homology.^{1,2} They perform important metabolic roles in our cells as they convert endogenous and exogenous aldehydes into carboxylic acids.³ ALDHs are nicotinamide adenine dinucleotide (NAD⁺) dependent enzymes and have a conserved catalytic cysteine and glutamic acid. The mechanism by which they convert their aldehyde substrates into a carboxylic acid is shown in **Fig. 1.1**.¹ The catalytic cysteine is activated by the neighboring glutamic acid residue via deprotonation. Nucleophilic attack of the catalytic cysteine on the aldehyde results in a hemithioacetal. Subsequent hydride abstraction by NAD⁺ results in a thioester. The thioester bond is hydrolyzed by a water molecule, which is deprotonated by the neighboring glutamate, thereby releasing the product.⁴ The conversion of aldehydes into carboxylic acids is vital as aldehydes are reactive functional groups, which may form adducts with both proteins and DNA leading to cellular damage.⁵

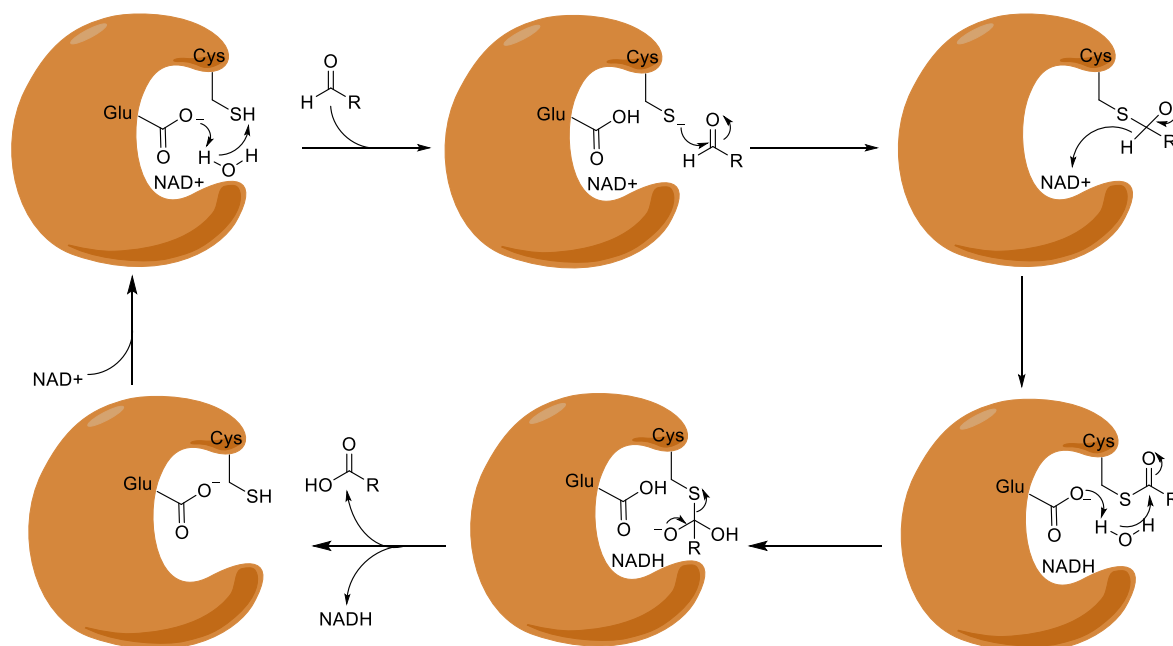


Fig. 1.1 | General enzymatic mechanism of ALDH enzymes. Starting from the upper left: the cofactor NAD^+ binds and the glutamate activates the catalytic cysteine via deprotonation. The substrate then binds to the enzyme, followed by nucleophilic attack of the catalytic cysteine on the aldehyde. Hydride abstraction by NAD^+ results in a thioester. Glutamate assisted hydrolysis of the thioester bond results in the release of the product and NADH .

ALDH activity is not only essential for the detoxification of aldehydes, but also for the production of several functional biomolecules, such as retinoic acid², γ -aminobutyric acid (GABA)⁶, folate⁷ and betaine⁸, that regulate important cellular processes.⁹ Not surprisingly, mutations in certain ALDH enzymes that compromise their enzymatic activity are associated with metabolic diseases¹⁰, such as Sjögren-Larsson syndrome¹¹, type II hyperprolinemia¹² and 4-hydroxybutyric aciduria.¹³ These are autosomal recessive disorders linked to mutations in *ALDH3A2*, *ALDH4A1* and *ALDH5A1*, respectively.^{11–13}

Upregulation of ALDH expression levels is commonly observed in cancer cells. ALDH activity in breast cancer has been linked with chemo- and radiotherapy resistance.¹⁴ For example, *ALDH1A1* overexpression is predictive of chemoresistance towards cyclophosphamide and the expression of *ALDH1A3* is associated with poor clinical outcome in breast cancer.^{15–19} Consequently, ALDH inhibition is a potential therapeutic strategy to overcome therapy resistance in cancer.

To date, the only ALDH inhibitor used in the clinic is disulfiram (Antabuse, **Fig. 1.2**), which is prescribed to support chronic alcoholics to maintain sobriety. The compound was originally used in industry as an accelerator of the rubber vulcanization process. It was first linked to ethanol metabolism in 1937 by

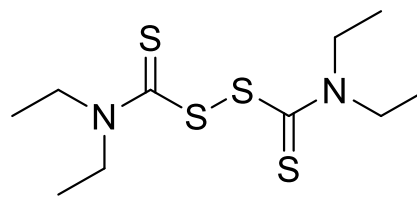


Fig. 1.2 | Chemical structure of disulfiram

E. Williams, who reported on the inability to consume alcohol of workers handling disulfiram.²⁰ Hald and Jacobsen showed that the sensitization to ethanol by disulfiram is accompanied by increased acetaldehyde concentrations in the blood.²¹ Although disulfiram has been shown to be a pan-ALDH inhibitor, its effect on ethanol metabolism has mainly be ascribed to its inhibition of mitochondrial ALDH2. ALDH2 is the main ALDH responsible for converting acetaldehyde, formed after the dehydrogenation of ethanol in the first metabolic step, into acetate in the liver.^{22,23}

Vitamin A: biological effects and metabolism

Vitamin A or retinol is taken up from the diet as the vitamin A precursors, β -carotene or retinyl esters.^{24,25} Retinyl esters are hydrolyzed into retinol in the intestinal lumen by pancreatic enzymes after which retinol is taken up by the intestinal epithelial cells.²⁶ Inside these cells, retinol can be re-esterified into retinyl esters and stored in blood circulating lipoprotein particles, called chylomicrons, which are transported to the liver, the main storage depot of vitamin A in the human body.²⁷ Alternatively, Retinol can be converted by the intestinal epithelial cells into retinal by retinol dehydrogenases (RDHs), which are members of the short chain dehydrogenase/reductase (SDR) superfamily²⁸, and alcohol dehydrogenases (ADHs).²⁹ The contrary reaction, reduction of retinal to retinol, can be performed by aldo-keto reductases (AKRs)³⁰ and RDHs.²⁸

β -carotene is taken up directly by intestinal epithelial cells and cleaved into two retinal molecules.³¹ Retinal is subsequently oxidized to retinoic acid by ALDHs in an irreversible step. A schematic overview of the enzymes involved in vitamin A metabolism in the small intestines is shown in **Fig. 1.3**.

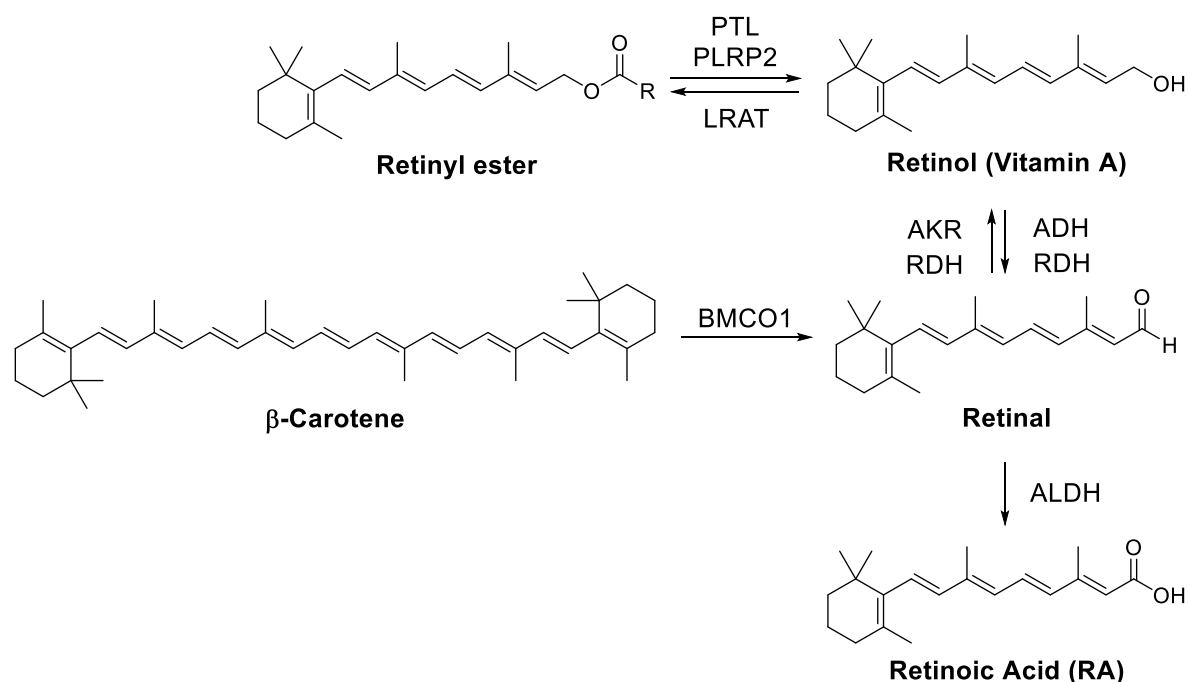


Fig. 1.3 | Schematic overview of vitamin A metabolism. Retinyl esters are converted in the lumen by pancreatic triglyceride lipase (PTL) and pancreatic lipase-related protein 2 (PLRP2) into retinol and then taken up by the intestinal epithelial cells (IECs). The IECs can convert retinol either back into retinyl esters intended for storage in the liver via lecithin retinol acyltransferase (LRAT) or into retinal by alcohol dehydrogenases (ADHs) and retinol dehydrogenases (RDHs). Retinal can be transformed back into retinol by aldo-keto reductases (AKRs) and RDHs. β -Carotene is cleaved by β -carotene 15,15'-dioxygenase (BCMO1) forming two molecules of retinal in the IECs. Retinal can subsequently be metabolized into retinoic acid by aldehyde dehydrogenases (ALDHs).

Retinoic acid regulates many cellular and physiological functions, including embryonic development, immunomodulation, neuronal differentiation and (cancer) stem cell proliferation.^{32–35} Most of these functions of retinoic acid are mediated via the retinoic acid receptor (RAR), which forms heterodimers with the retinoid X receptor (RXR). Binding of retinoic acid to the RAR/RXR heterodimer complex modulates gene transcription by recruiting different cofactors to the DNA-bound complex in a cell specific manner.^{36,37} Retinoic acid is essential in chordate animals as disruption of retinoic acid signaling can lead to severe (neural) developmental defects, autoimmunity disorders and cancer.^{32,38–40} The key function of retinoic acid in biological signaling implies that its cellular levels are tightly regulated.

Three retinaldehyde dehydrogenases (ALDH1A1, ALDH1A2 and ALDH1A3) have been identified that produce retinoic acid.^{1,2} These ALDHs have a variable and inducible cellular expression pattern. Their activity is regulated by post-translational modifications.^{41,42} Immunoblotting and quantitative real-time polymerase chain reaction (RT-PCR) are currently used to determine retinaldehyde dehydrogenase expression levels in cells, but these assays report solely on protein expression levels and not on activity.^{16,43}

The ALDEFLUOR assay does report on global ALDH activity levels in (cancer) stem cells. This assay uses a fluorescent aldehyde that upon oxidation to a charged fluorescent carboxylate is trapped within the cell. Therefore, cells with high ALDH activity become fluorescent and can be detected using fluorescence activated cell sorting (FACS) (**Fig. 1.4**).

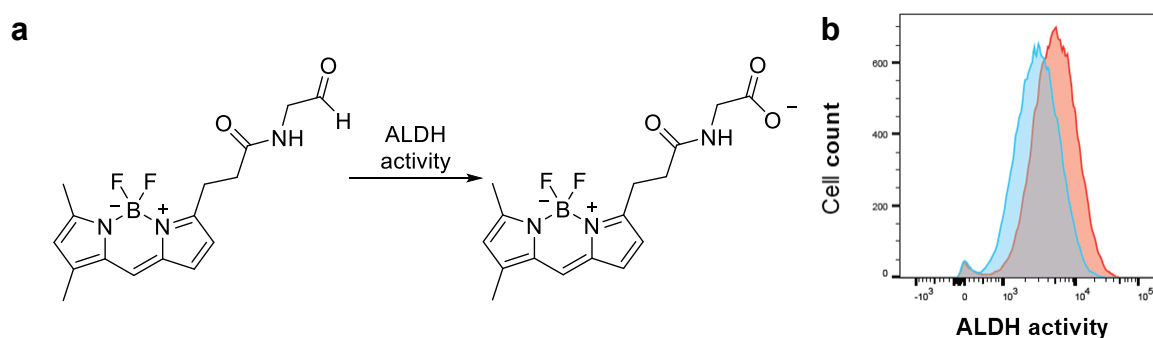


Fig. 1.4 | Determination of ALDH activity using the ALDEFLUOR assay. **a**, Chemical representation of the conversion of the ALDEFLUOR substrate by ALDHs. **b**, Representative FACS plot of cells treated with ALDEFLUOR (red) and a negative control in which cells were pretreated with an ALDH inhibitor (blue).

However, the ALDEFLUOR assay does not discriminate between individual ALDHs.⁴⁴ Recently developed selective fluorescent substrates report on the activity of a single enzyme, but do not provide an overview of the global ALDH activity present in a biological system.^{45,46} The development of chemical tools and methods to profile the levels of active retinaldehyde dehydrogenases is, therefore, important to study retinoic acid formation in health and disease, and for the discovery of effective ALDH inhibitors for therapeutic strategies.

Activity-based protein profiling

Activity-based protein profiling (ABPP) is a powerful chemical biological method that is ideally suited to report on the abundance and identity of active enzymes in complex biological systems.⁴⁷ It employs activity-based probes (ABP) that covalently and irreversibly react with conserved catalytic amino acid residues in the active site of proteins. ABPs constitute an electrophilic warhead to covalently react with the catalytic nucleophile, a recognition element for enzyme affinity and in the case of a two-step probe a ligation handle for the introduction of reporter molecules, such as fluorophores or biotin. These reporter groups enable target visualization by resolving samples on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by in-gel fluorescent scanning or target identification by affinity enrichment and chemical proteomics, respectively (**Fig. 1.5**).

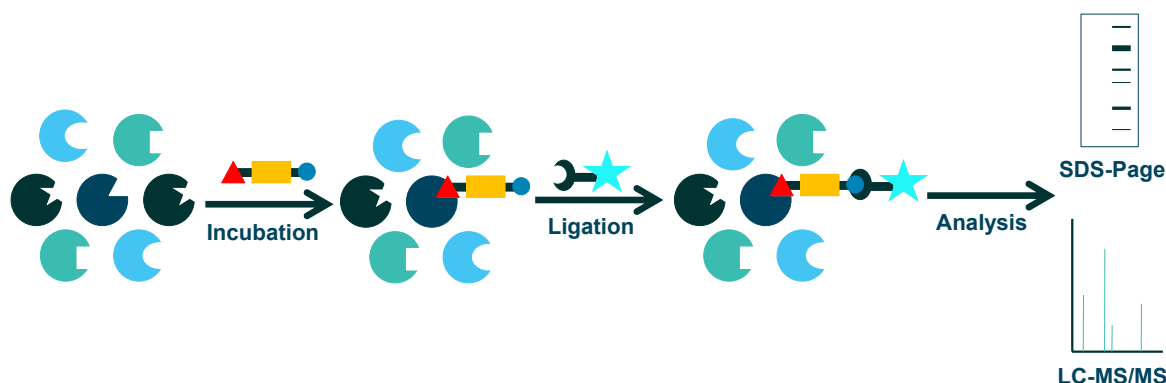


Fig. 1.5 | Activity-based Protein Profiling. Schematic representation of an ABPP workflow using a two-step probe.

Commonly used ligation handles are alkynes and azides which are coupled to the reporter groups using a copper(I)-catalyzed azide-alkyne [2+3] cycloaddition.⁴⁸ The introduction of the reporter group after covalent interaction of the probe with its target offers the advantage of avoiding potential issues with potency, selectivity and cell permeability.⁴⁹

There are two main applications for which ABPs are commonly used: comparative and competitive ABPP. Comparative ABPP studies the abundance of active enzymes in biological samples derived from different biological states, such as healthy versus disease states^{50,51}, different differentiation states, developmental stages or from various tissues.^{52,53} Competitive ABPP reports on the potency and selectivity of inhibitors. Preincubation of biological samples with an inhibitor blocks the active site of interacting enzymes, which can

therefore, not be labeled by the ABP. This results in a concentration-dependent decrease in labeled proteins, which can be quantified using chemical proteomics. ABPP can be applied in a wide variety of biological samples ranging from cell lysate and living cells to *in vivo* animal models.⁵⁴

ABPP has been successfully and widely applied in the field of serine hydrolases^{52,55}, proteases⁵⁶, kinases⁵⁷ and glycosidases.⁵⁸ However, only a few ABPs for oxidoreductases, which target P450⁵⁹, MAO⁶⁰ and 15-LOX⁶¹, have been published. There are currently no ABPs that report on ALDH activity. It is envisioned that chemical tools and methods that allow the identification and quantitation of the levels of active ALDHs in human cells is of utility to study the role of these enzymes in health and disease.

Aim and outline

The aim of this thesis is to design, synthesize and apply activity-based probes for the profiling of aldehyde dehydrogenase activity in complex biological samples. The outline of this thesis is as follows:

Chapter 2 provides an overview of the currently available lipid-based ABPs and discusses the (future) applications of lipid-based probes in immunology.

Chapter 3 describes the design and synthesis of the first-in-class retinal-based ABP **LEI-945** for the profiling of ALDHs.

Chapter 4 focusses on the biological validation of **LEI-945** as an ABP for ALDHs. The detection of various ALDH isozymes by **LEI-945** was demonstrated in living cells using fluorescence and chemical proteomic approaches.

Chapter 5 describes the utility of **LEI-945** in quantitating ALDH isozyme activity in a panel of breastcancer subtypes. **LEI-945** was superior to the widely used ALDEFLUOR assay in explaining the ability of breast cancer (stem) cells to produce retinoic acid. Furthermore, the probe revealed the cellular selectivity profile of an advanced ALDH1A1 inhibitor, thereby prompting the investigation of its cytotoxic nature.

Chapter 6 elaborates on the role of vitamin A metabolism in intestinal epithelial cells in immune homeostasis. Comparative ABPP using **LEI-945** of intestinal cells isolated from wild type and ALDH1A1 knockout mice pointed to ALDH1B1 as a potential alternative retinoic acid producing enzyme.

Chapter 7 describes the synthesis of alkyne functionalized retinoid analogues without an electrophilic warhead. Clickable retinoic acid differentiated HL60 cells into granulocytes in a similar fashion as retinoic acid. The probes were subsequently used in photoaffinity based chemical proteomics to define the retinoid interactome.

Chapter 8 describes the design, synthesis and application of an easily accessible broadspectrum ABP with a non-retinoid scaffold in the profiling of ALDH activity in cancer cells.

Chapter 9 summarizes the work presented in this thesis and reflects on future opportunities for the application of chemical probes profiling aldehyde dehydrogenases and retinoid interacting partners.

References

1. Koppaka, V. *et al.* Aldehyde Dehydrogenase Inhibitors: a Comprehensive Review of the Pharmacology, Mechanism of Action, Substrate Specificity, and Clinical Application. *Pharmacol. Rev.* **64**, 520–539 (2012).
2. Duester, G., Mic, F. A. & Molotkov, A. Cytosolic retinoid dehydrogenases govern ubiquitous metabolism of retinol to retinaldehyde followed by tissue-specific metabolism to retinoic acid. in *Chemico-Biological Interactions* **143–144**, 201–210 (Elsevier, 2003).
3. Vasiliou, V., Pappa, A. & Petersen, D. R. Role of aldehyde dehydrogenases in endogenous and xenobiotic metabolism. *Chem. Biol. Interact.* **129**, 1–19 (2000).
4. Wang, X. & Weiner, H. Involvement of Glutamate 268 in the Active Site of Human Liver Mitochondrial (Class 2) Aldehyde Dehydrogenase as Probed by Site-Directed Mutagenesis. *Biochemistry* **34**, 237–243 (1995).
5. Setshedi, M., Wands, J. R. & De La Monte, S. M. Acetaldehyde adducts in alcoholic liver disease. *Oxidative Medicine and Cellular Longevity* **3**, 178–185 (2010).
6. Kurys, G., Ambroziak, W. & Pietruszkos, R. Human Aldehyde Dehydrogenase. *J. Biol. Chem.* **264**, 4715–4721 (1989).
7. Kutzbach, C. & Stokstad, E. L. R. Mammalian methylenetetrahydrofolate reductase Partial purification, properties, and inhibition by S-adenosylmethionine. *BBA - Enzymol.* **250**, 459–477 (1971).
8. Chern, M. K. & Pietruszko, R. Human aldehyde dehydrogenase E3 isozyme is a betaine aldehyde dehydrogenase. *Biochem. Biophys. Res. Commun.* **213**, 561–568 (1995).
9. Jackson, B. *et al.* Update on the aldehyde dehydrogenase gene (ALDH) superfamily. *Hum. Genomics* **5**, 283–303 (2011).
10. Vasiliou, V. & Pappa, A. Polymorphisms of Human Aldehyde Dehydrogenases. *Pharmacology* **61**, 192–198 (2000).
11. Laurenzi, V. De *et al.* Sjögren–Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene. *Nat. Genet.* **12**, 52–57 (1996).
12. Geraghty, M. T. *et al.* Mutations in the $\Delta 1$ -pyrroline 5-carboxylate dehydrogenase gene cause type II hyperprolinemia. *Hum. Mol. Genet.* **7**, 1411–1415 (1998).
13. Chambliss, K. L. *et al.* Two Exon-Skipping Mutations as the Molecular Basis of Succinic Semialdehyde Dehydrogenase Deficiency (4-Hydroxybutyric Aciduria). *Am. J. Hum. Genet.* **63**, 399–408 (1998).
14. Croker, A. K. & Allan, A. L. Inhibition of aldehyde dehydrogenase (ALDH) activity reduces chemotherapy and radiation resistance of stem-like ALDH hiCD44 + human breast cancer cells. *Breast Cancer Res. Treat.* **133**, 75–87 (2012).
15. Qiu, Y. *et al.* The expression of aldehyde dehydrogenase family in breast cancer. *J. Breast Cancer* **17**, 54–60 (2014).
16. Marcato, P. *et al.* Aldehyde Dehydrogenase Activity of Breast Cancer Stem Cells Is Primarily Due To Isoform ALDH1A3 and Its Expression Is Predictive of Metastasis. *Stem Cells* **29**, 32–45 (2011).
17. Sládek, N. E., Kollander, R., Sreerama, L. & Kiang, D. T. Cellular levels of aldehyde dehydrogenases (ALDH1A1 and ALDH3A1) as predictors of therapeutic responses to cyclophosphamide-based chemotherapy of breast cancer: A retrospective study. *Cancer Chemother. Pharmacol.* **49**, 309–321 (2002).
18. Tomita, H., Tanaka, K., Tanaka, T. & Hara, A. Aldehyde dehydrogenase 1A1 in stem cells and cancer. *Oncotarget* **7**, 11018–32 (2016).
19. De Beca, F. F. *et al.* Cancer stem cells markers CD44, CD24 and ALDH1 in breast cancer special histological types. *J. Clin. Pathol.* **66**, 187–191 (2013).
20. Williams, E. E. Effects of Alcohol on Workers with Carbon Disulfide. *Journal of the American Medical Association* **109**, 1472–1473 (1937).
21. Hald, J. & Jacobsen, E. The Formation of Acetaldehyde in the Organism after Ingestion of Antabuse (Tetraethylthiuramdisulphide) and Alcohol. *Acta Pharmacol. Toxicol. (Copenh).* **4**, 305–310 (1948).
22. Yasgar, A. *et al.* A High-Content assay enables the automated screening and identification of small molecules with specific ALDH1A1-Inhibitory activity. *PLoS One* **12**, 1–19 (2017).
23. Chen, C.-H., Ferreira, J. C. B., Gross, E. R. & Mochly-Rosen, D. Targeting Aldehyde Dehydrogenase 2: New Therapeutic Opportunities. *Physiol. Rev.* **94**, 1–34 (2014).
24. D’Ambrosio, D. N., Clugston, R. D. & Blaner, W. S. Vitamin A metabolism: An update. *Nutrients* **3**, 63–103 (2011).
25. Woollard, G. Retinol, retinoic acid, carotenes and carotenoids: Vitamin a structure and terminology. *Food Nutr. Components Focus* **1**, 3–22 (2012).
26. Harrison, E. H. Mechanims of digestion and absorption of dietary vitamin A. *Annu. Rev. Nutr.* **25**, 87–103 (2005).

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27. Blomhoff, R., Green, M. H., Green, J. B., Berg, T. & Norum, K. R. Vitamin A metabolism: new perspectives on absorption, transport, and storage. *Physiol.Rev.* **71**, 951–990 (1991).
28. Lidén, M. & Eriksson, U. Understanding retinol metabolism: Structure and function of retinol dehydrogenases. *J. Biol. Chem.* **281**, 13001–13004 (2006).
29. Molotkov, A. *et al.* Stimulation of retinoic acid production and growth by ubiquitously expressed alcohol dehydrogenase Adh3. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 5337–5342 (2002).
30. Ruiz, F. X., Porté, S., Parés, X. & Farrés, J. Biological role of aldo-keto reductases in retinoic acid biosynthesis and signaling. *Front. Pharmacol.* **3**, 58 (2012).
31. D’Ambrosio, D. N., Clugston, R. D. & Blaner, W. S. Vitamin A Metabolism: An Update. *Nutrients* **3**, 63–103 (2011).
32. Zile, M. H. Vitamin A and Embryonic Development: An Overview. *J. Nutr.* **128**, 455–458 (1998).
33. Wiseman, E. M., Bar-El Dadon, S. & Reifen, R. The vicious cycle of vitamin a deficiency: A review. *Crit. Rev. Food Sci. Nutr.* **57**, 3703–3714 (2017).
34. Mora, J. R., Iwata, M. & Andrian, U. H. Von. Vitamin effects on the immune system. *Nat. Rev. Immunol.* **8**, 685–698 (2008).
35. Blomhoff, H. K. *et al.* Vitamin A is a key regulator for cell growth, cytokine production, and differentiation in normal B cells. *J. Biol. Chem.* **267**, 23988–23992 (1992).
36. Carlberg, C. Lipid soluble vitamins in gene regulation. *BioFactors* **10**, 91–97 (1999).
37. Balmer, J. E. & Blomhoff, R. Gene expression regulation by retinoic acid. *J. Lipid Res.* **43**, 1773–808 (2002).
38. Noy, N. Between Death and Survival: Retinoic Acid in Regulation of Apoptosis. *Annu. Rev. Nutr.* **30**, 201–17 (2010).
39. Schambach, F., Schupp, M., Lazar, M. A. & Reiner, S. L. Activation of retinoic acid receptor- α favours regulatory T cell induction at the expense of IL-17-secreting T helper cell differentiation. *Eur. J. Immunol.* **37**, 2396–2399 (2007).
40. Erkelens, M. N. & Mebius, R. E. Retinoic Acid and Immune Homeostasis: A Balancing Act. *Trends in Immunology* **38**, 168–180 (2017).
41. Wang, J. *et al.* Phosphorylation-dependent regulation of ALDH1A1 by Aurora kinase A: Insights on their synergistic relationship in pancreatic cancer. *BMC Biol.* **15**, 1–22 (2017).
42. Zhao, D. *et al.* NOTCH-induced aldehyde dehydrogenase 1A1 deacetylation promotes breast cancer stem cells. *J. Clin. Invest.* **124**, 5453–5465 (2014).
43. Moreb, J. S., Zucali, J. R., Ostmark, B. & Benson, N. A. Heterogeneity of aldehyde dehydrogenase expression in lung cancer cell lines is revealed by Aldefluor flow cytometry-based assay. *Cytom. Part B Clin. Cytom.* **72B**, 281–289 (2007).
44. Zhou, L. *et al.* Identification of cancer-type specific expression patterns for active aldehyde dehydrogenase (ALDH) isoforms in ALDEFUOR assay. *Cell Biol. Toxicol.* **35**, 161–177 (2019).
45. Anorma, C. *et al.* Surveillance of Cancer Stem Cell Plasticity Using an Isoform-Selective Fluorescent Probe for Aldehyde Dehydrogenase 1A1. *ACS Cent. Sci.* **4**, 1045–1055 (2018).
46. Yagishita, A. *et al.* Development of Highly Selective Fluorescent Probe Enabling Flow-Cytometric Isolation of ALDH3A1-Positive Viable Cells. *Bioconjug. Chem.* **28**, 302–306 (2017).
47. Cravatt, B. F., Wright, A. T. & Kozarich, J. W. Activity-Based Protein Profiling: From Enzyme Chemistry to Proteomic Chemistry. *Annu. Rev. Biochem.* **77**, 383–414 (2008).
48. Hein, J. E. & Fokin, V. V. Copper-catalyzed azide-alkyne cycloaddition (CuAAC) and beyond: New reactivity of copper(I) acetylides. *Chemical Society Reviews* **39**, 1302–1315 (2010).
49. Janssen, A. P. A. *et al.* Development of a Multiplexed Activity-Based Protein Profiling Assay to Evaluate Activity of Endocannabinoid Hydrolase Inhibitors. *ACS Chem. Biol.* **13**, 2406–2413 (2018).
50. van Rooden, E. J. *et al.* Chemical proteomic analysis of serine hydrolase activity in Niemann-Pick Type C mouse brain. *Front. Neurosci.* **12**, 440 (2018).
51. Nomura, D. K., Dix, M. M. & Cravatt, B. F. Activity-based protein profiling for biochemical pathway discovery in cancer. *Nature Reviews Cancer* **10**, 630–638 (2010).
52. Liu, Y., Patricelli, M. P. & Cravatt, B. F. Activity-based protein profiling: The serine hydrolases. *Proc. Natl. Acad. Sci.* **96**, 14694–14699 (1999).
53. Baggelaar, M. P. *et al.* Chemical Proteomics Maps Brain Region Specific Activity of Endocannabinoid Hydrolases. *ACS Chem. Biol.* **12**, 852–861 (2017).
54. Van Esbroeck, A. C. M. *et al.* Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science* **356**, 1084–1087 (2017).

55. Baggelaar, M. P. *et al.* Development of an Activity-Based Probe and In Silico Design Reveal Highly Selective Inhibitors for Diacylglycerol Lipase- α in Brain. *Angew. Chemie Int. Ed.* **52**, 12081–12085 (2013).
56. Serim, S., Haedke, U. & Verhelst, S. H. L. Activity-Based Probes for the Study of Proteases: Recent Advances and Developments. *ChemMedChem* **7**, 1146–1159 (2012).
57. Zhao, Q. *et al.* Broad-spectrum kinase profiling in live cells with lysine-targeted sulfonyl fluoride probes. *J. Am. Chem. Soc.* **139**, 680–685 (2017).
58. Kuo, C. L. *et al.* Activity-Based Probes for Glycosidases: Profiling and Other Applications. in *Methods in Enzymology* **598**, 217–235 (Academic Press, 2018).
59. Wright, A. T. & Cravatt, B. F. Chemical Proteomic Probes for Profiling Cytochrome P450 Activities and Drug Interactions In Vivo. *Chem. Biol.* **14**, 1043–1051 (2007).
60. Krysiak, J. M. *et al.* Activity-based probes for studying the activity of flavin-dependent oxidases and for the protein target profiling of monoamine oxidase inhibitors. *Angew. Chemie Int. Ed.* **51**, 7035–7040 (2012).
61. Eleftheriadis, N., Thee, S. A., Zwinderman, M. R. H., Leus, N. G. J. & Dekker, F. J. Activity-Based Probes for 15-Lipoxygenase-1. *Angew. Chemie Int. Ed.* **55**, 12300–12305 (2016).

