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Antibody-antigen complex structures



ANTIBODY-ANTIGEN
STRUCTURES



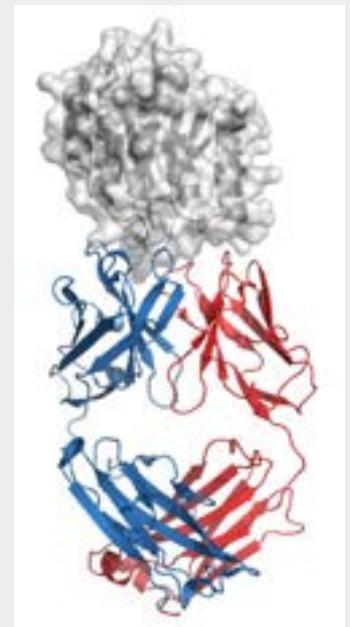
Fab-antigen Structures

Don't work in the dark!

Access to structural information increases your understanding
and enables you to execute projects faster.

Use structural information for:

- ▶ Epitope definition to file stronger IP
- ▶ Understanding MoA
- ▶ Structure-based design
- ▶ Structural characterization of protein drugs (HOS)
- ▶ Antibody engineering: affinity maturation
- ▶ Antibody engineering: humanization
- ▶ Antibody engineering: ADC

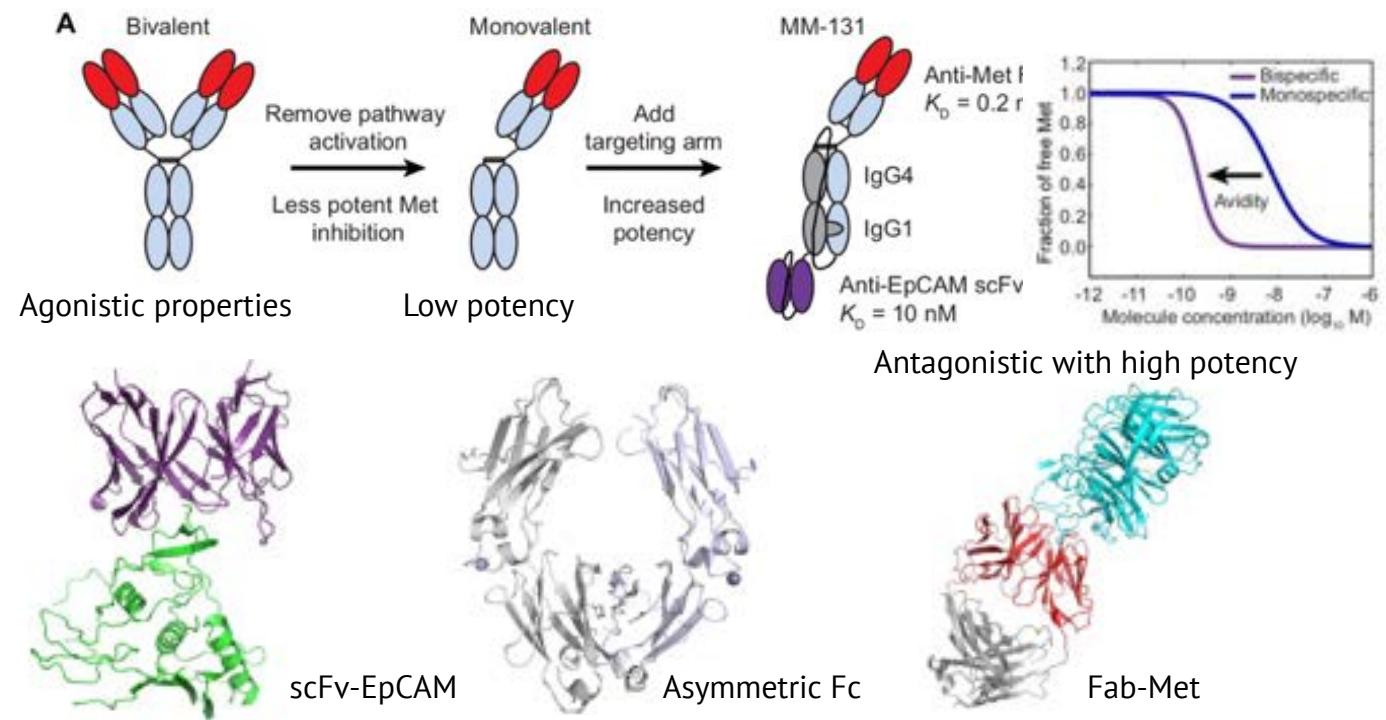




MM-131 – Antigen Structures Case Study

Client project: Bispecific anti-Met/EpCAM mAb MM-131 in complex with its antigens

Collaboration with **Merrimack Pharmaceuticals**, Cambridge, MA



Published in PNAS!

PDB codes: 6I07, 6HYG, 6I04

Casaletto *et al.*, 2019, PNAS, 116, 7533-7542.



Davoceticept (ALPN-202) - An engineered CD80 variant fusion therapeutic

Client project: ALPN-202 in complex with PD-L1

Collaboration with **Alpine Immune Sciences**, Seattle, WA

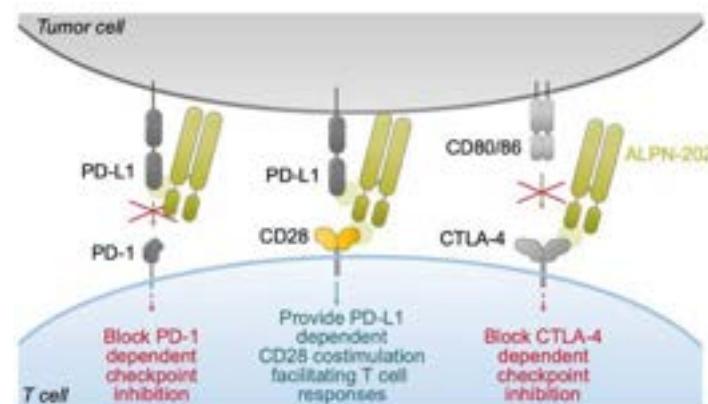
ARTICLE

The engineered CD80 variant fusion therapeutic davoceticept combines checkpoint antagonism with conditional CD28 costimulation for anti-tumor immunity

Mark A. Maurer^{1,2*}, Katherine S. Lewis², Joseph L. Kusser², Sean Anderson², Chelsea L. Giugno², Sébastien Chardoulet^{2,3}, Steven L. Muir², Kyle M. Klein², Chris Nease^{2,4}, Martin F. Reinhold², Mark W. Krouse², Ryan Sauerhoff², Nancy R. Dillen², Steven D. Lau^{2,5}, Yvonne Raymond Kuehne², Masato Matsui^{2,6}, Derek T. Loprinzi², Bahin Watson², Kristine M. Sestakova^{2,7} & Michael L. Popp^{2,8}

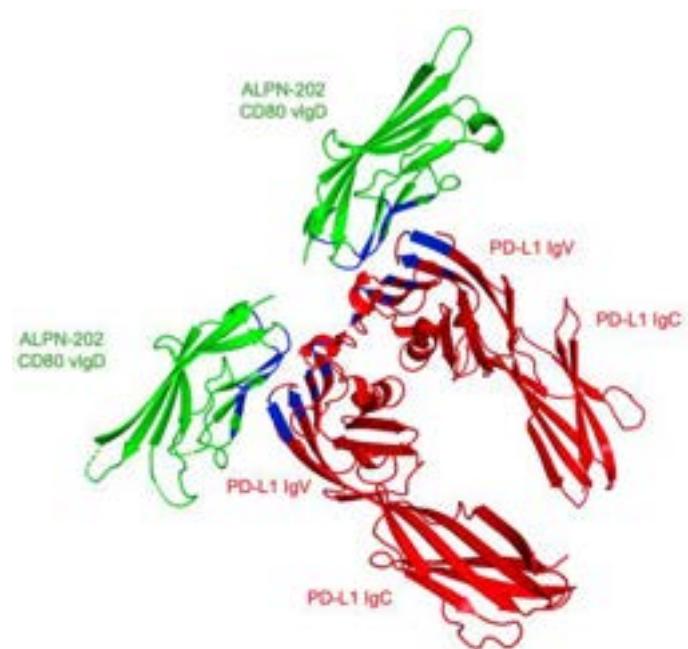
*Equal contribution. ¹Fluigent Communications Inc., San Leandro, CA, USA. ²Alpine Immune Sciences, Inc., Seattle, WA, USA. ³University of Washington, Seattle, WA, USA. ⁴Seattle Children's Research Institute, Seattle, WA, USA. ⁵University of Washington, Seattle, WA, USA. ⁶University of California, Los Angeles, CA, USA. ⁷University of Colorado, Boulder, Boulder, CO, USA. ⁸University of Colorado, Denver, Aurora, CO, USA.

Despite the recent clinical success of T cell checkpoint inhibition targeting the CTLA-4 and PD-1 pathways, many patients either fail to achieve objective responses or they develop resistance to therapy. In some cases, poor responses to checkpoint blockade have been linked to suboptimal CD28 costimulation and the inability to generate and maintain a productive adaptive T cell immune response. To address this need, we utilize directed evolution to engineer a CD80 variant with increased PD-1 affinity and have this to an immunoprecipitable T cell domain, creating a therapeutic (ALPN-202, davoceticept) capable of providing CD28 costimulation in a PD-1-dependent fashion while also antagonizing PD-1/PD-1 and CTLA-4/CD137/CD28 interactions. We demonstrate that by combining CD28 stimulation and dual checkpoint inhibition, ALPN-202 unleashes T cell activation and anti-tumor efficacy in cell-based assays and mouse tumor models more powerfully than checkpoint blockade alone and thus has the potential to generate potent, clinically meaningful anti-tumor immunity in humans.



The three mechanisms of action of ALPN-202:

- Blockade of PD-1–PD-L1 interaction
- PD-L1-dependent CD28 costimulation
- Blockade of CTLA-4–CD80/CD86 interactions.



X-ray structure of ALPN-202 CD80 vlgD in complex with PD-L1



Structural basis of activation and antagonism of receptor signaling mediated by interleukin-27

Client project: SRF388 Fab in complex IL-27

Collaboration with **Surface Oncology**, Cambridge, MA

Cell Reports

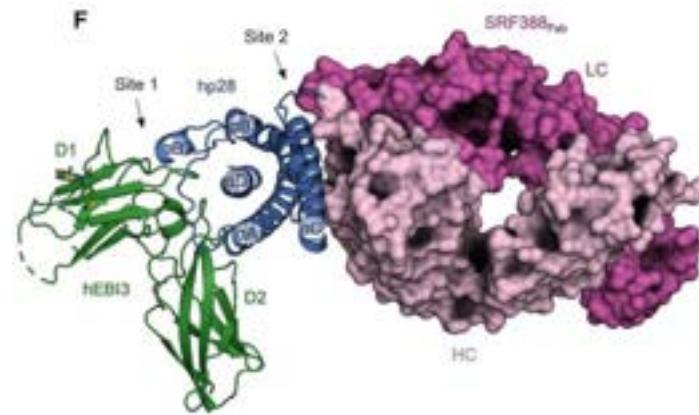


Structural basis of activation and antagonism of receptor signalling mediated by interleukin-5

10

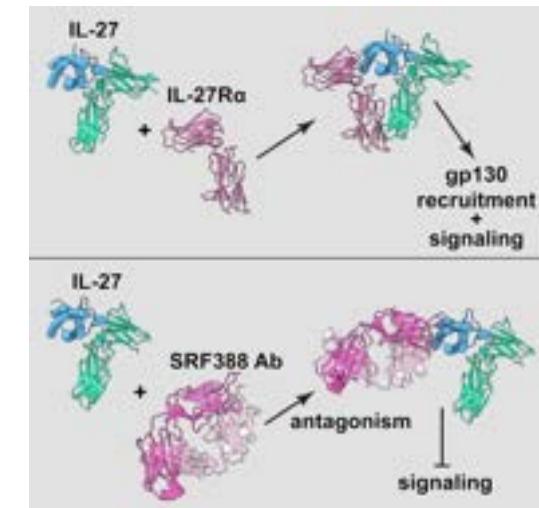
ANSWER

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X-ray structure of SRF388 Fab in complex with IL-27

PDB code: 7ZXK



- IL-27Ra interacts both with the p28 and EBI3 subunits of IL-27
 - SRF388 and IL-27Ra occupy mutually exclusive binding sites on IL-27
 - IL-27 mediates receptor assemblies distinct from IL-12 and IL-23

Skladanowska et al., 2022, Cell Reports, 41, 111490



Activin ligand trap

Client project: ActRIIB-Alk4-Fc in complex with activin A and anti-ActRIIB Fab

Collaboration with **Acceleron Pharma**, Cambridge, MA

iScience

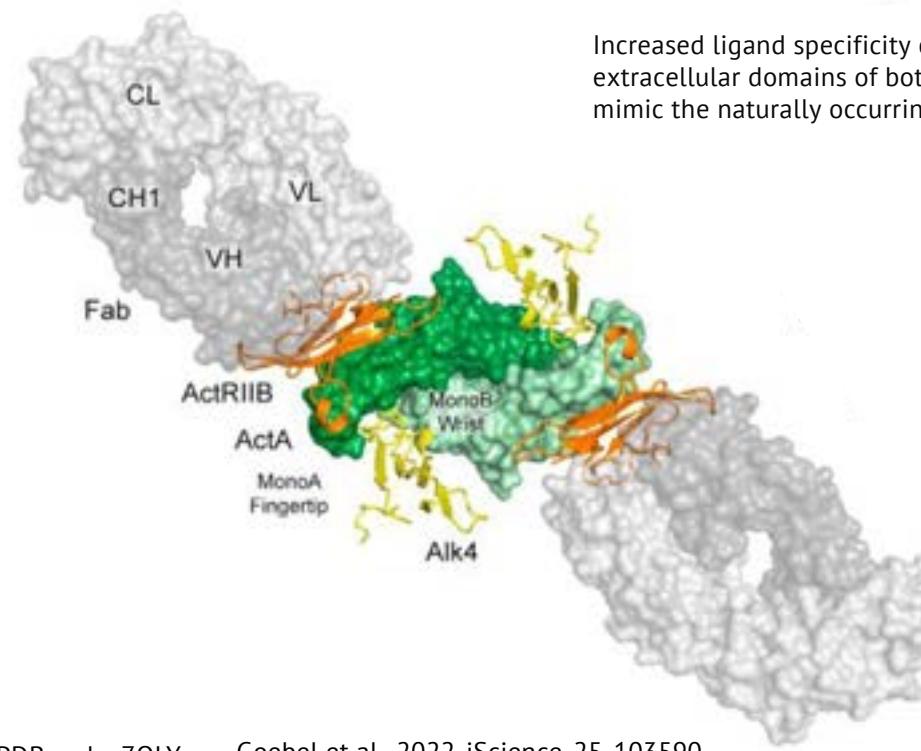
Article

Structures of activin ligand traps using natural sets of type I and type II TGF β receptors

Enrich J. Goebel, Chandan Kumar, Katerina M. Gregory, ..., Rosalyn C. Cambengue, Ravinder Kumar, Thomas E. Thompson

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The figure shows a schematic of the Activin Ligand Trap mechanism where Activin Ligands bind to the ActRIIB-Alk4-Fc fusion receptor, inhibiting Smad2/3 signal transduction. Below is a ribbon diagram of the complex between Activin A (green), ActRIIB (grey), Alk4 (orange), and an anti-ActRIIB Fab (yellow).



Increased ligand specificity can be accomplished by using the extracellular domains of both the type I and type II receptor to mimic the naturally occurring signaling complex.

Structure of ActA/ActRIIB:
Alk4/anti-ActRIIB Fab complex

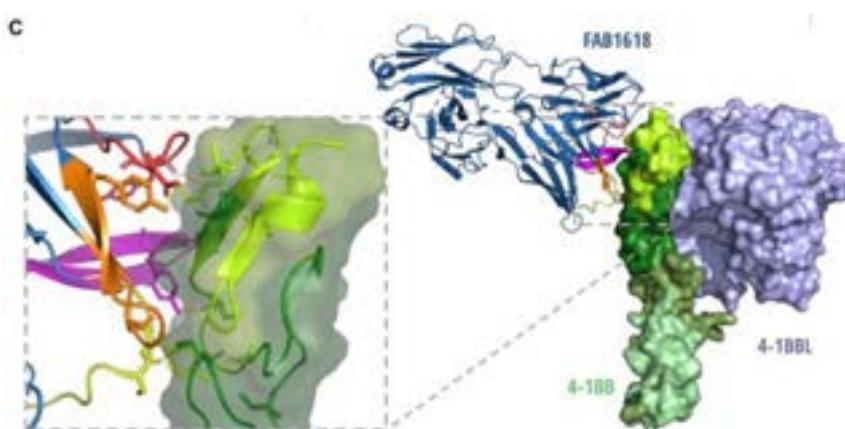


The bispecific 4-1BB x 5T4 agonist, ALG.APV-527, mediates strong T cell activation and potent anti-tumor activity

Client project: ALG.APV-527 (Fab1618) in complex with 4-1BB (CD137)

Collaboration with Alligator Bioscience, Lund, Sweden

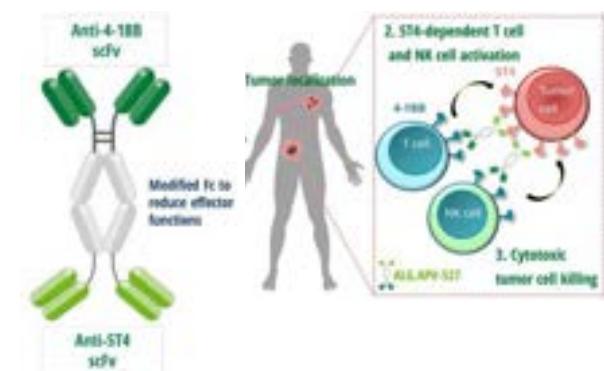
MOLECULAR CANCER THERAPEUTICS



X-ray structure of Fab1618 in complex with 4-1BB

PDB code: 7YXU

Nelson et al., 2022, Mol. Cancer Ther., 22-0395.



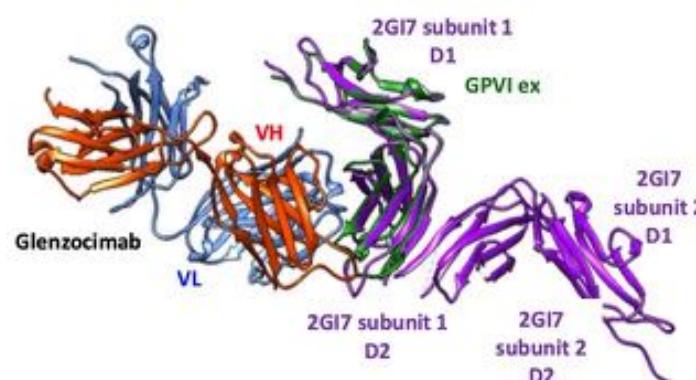
- ALG.APV-527 directs the stimulation of T cells and NK cells to 5T4+ tumors and is designed to minimize the toxicity observed with other 4-1BB therapeutics
 - Binding sites of ALG.APV-527 and the 4-1BBL on 4-1BB are distinct



Targeting platelet GPVI with glenzocimab: a novel mechanism for inhibition

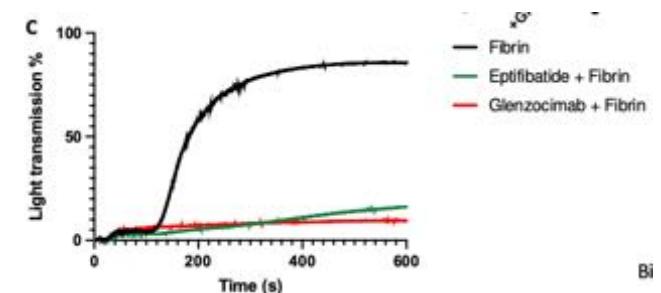
Client project: Glanzocimab Fab in complex with platelet glycoprotein VI

Collaboration with **Acticor Biotech**, Paris, France



X-ray structure of glenzocimab in complex with GPV

PDB code: 7R58



Glenzocimab inhibits fibrin-induced platelet aggregation

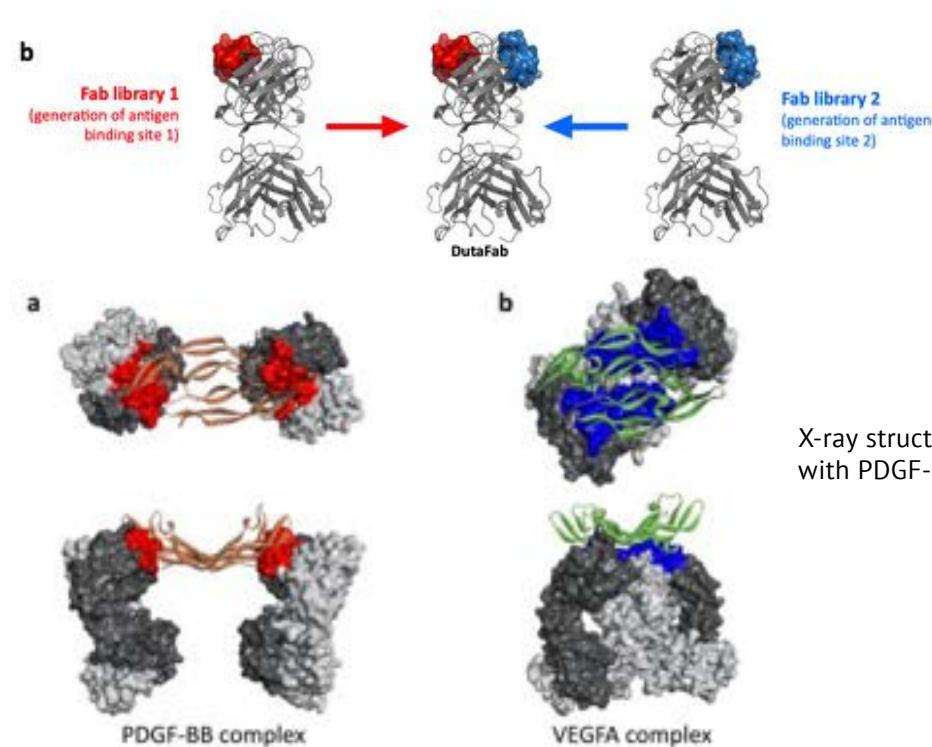
- GPVI binding to vascular collagen initiates thrombus formation and GPVI interactions with fibrin promote the growth and stability of the thrombus.
 - Crystal structure information enables the **elucidation of a novel mechanism** for the powerful anti thrombotic effect of glenzocimab, in which both ligands are blocked through a combination of steric hindrance and structural change.



DutaFabs - engineered Fab's that bind two antigens simultaneously

Client project: DutaFab (**Roche**) in complex with its antigens PDGF and VEGFA

The screenshot shows the article title, authors (Roland Beckmann, Kristian Jerschow, Valentin Fenn, Janina Speck, Kathrin Krause, Annette Meier, Melanie Röhl, Sascha Fäuerle, Raymond Künzli, Derek T. Logan, Martin Siegrist, & Hubert Kettenberger), and a brief abstract. The abstract discusses the development of dual-targeting Fab (DutaFab) molecules that combine two paratopes separated by independent binding sites within the human prebody C54 loop. It highlights the generation of antigen-binding sites 1 (C54R1, C54R2, and C54R3) and site 2 (C54R4, C54R5, and C54R6). Both paratopes can be independently selected and combined into the same DutaFab molecule. The study demonstrates that DutaFabs are able to bind two target molecules simultaneously at the same low-nanomolar IC₅₀ concentrations. In the present study, this platform is applied to generate DutaFab specific for VEGFR and PDGF-BB, which show high affinities, pharmacological stability, as well as superior efficacy over anti-VEGFR monoclonal antibodies. These molecules exemplify the potential of DutaFabs as a distinct class of antibody therapeutics, which is currently being evaluated in patients.



The DutaFab concept of separating paratopes on a single Fab

X-ray structure of the DutaFab in complex with PDGF-BB dimer and VEGFA dimer

Published in Nature Communications!

PDB code: 6T9E

6T9D

Beckmann et al., 2021, Nat Comm, 12:708.



Dusquetide modulates innate immune response through binding to p62

Client project: Dusquetide in complex with p62 (SQSTM1) ZZ domain

Collaboration with Soligenix, Princeton, NJ

Structure

e CellPress

Articles

Dusquetide modulates innate immune response through binding to p62

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SUMMARY

RS23389 (p62) is an autophagic receptor that plays a major role in mediating stress and innate immune responses. Previous studies reported p62 as a target of the proinflammatory defense response (DIR), however, the molecular mechanism of this process remains unclear. Here, we describe the structural basis and thermodynamic details of the association of p62 with the heat-generating protein (HGP), also known as the electron-transport chain (ETC)-coupling protein (CCP). Our results show that the HGP binds to the C-terminal domain of p62. This effect is due to electrostatic interactions between the carboxy-terminal domain of p62 and CCP. The CCP-induced association with p62 is reversible. Electrophoretic mobility shift assays (EMSA) and fluorescence resonance energy transfer (FRET) experiments show that the HGP enhances binding of the p62-HGP complex, increases p62 phosphorylation, and enhances G3BP1 assembly without activating autophagy. Our findings provide molecular details underlying the DIR action that may help in the development of new strategies to pharmacologically target p62.

INTRODUCTION

The major autophagy receptor p62 (also known as SQSTM1) has been implicated in immunological and inflammatory diseases such as sepsis, multiple sclerosis, and neurodegenerative disorders [1–4]. p62 functions as a signaling hub that mediates cell proliferation, growth, and survival and is required for recycling of cellular organelles. Accumulation of p62 promotes autophagy and cell death [5]. p62 is a key regulator of autophagy and maintains protein quality control, activation of mTORC1, protection of cells from stress-induced cell death, metabolism reprogramming, and degradation of excess cellular components [6–10].

p62 is a protein that contains an N-terminal RFP domain, including a C2-like loop (also known as CCP domain) [11]. Various binding partners of p62 have been identified, such as the membrane-interacting protein 1 (MIP-1), actin-interconversion protein 1 (AIP-1), nuclear interconversion protein 1 (NIP-1), and the p62-binding protein (P62BP) [12–15]. Binding of AIP-1 to p62S200 promotes the association with RFP and stimulates the AMPK/NF- κ B activity [7]. In addition, p62BP interacts with p62 and inhibits its nuclear localization [13]. p62S200 also mediates its nuclear regulation via RFP protein degradation signal and plays a central role

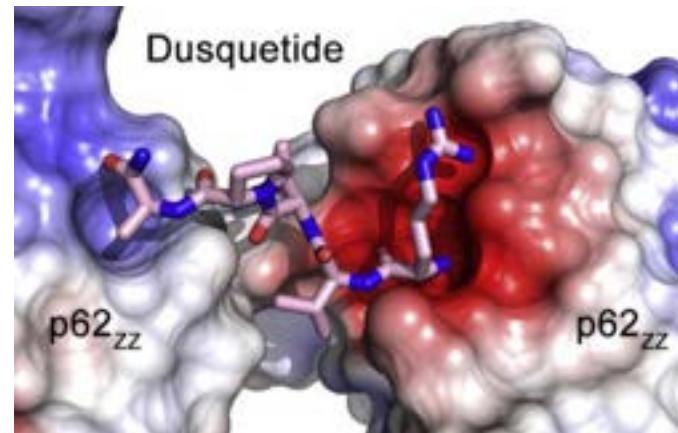
in activation of stress-induced selective autophagy [16–18] (see Fig. 1).

Owing to its role in modulating innate immune responses mediated in infected macrophages, p62 has emerged as a promising target for therapeutic intervention [19]. Recent studies show that DIR-1 or p62S200, induced based on natural microvesicle particles, regulates heat response, prevents tissue damage and increases host defense following infection [20–22]. Moreover, immunotherapy, an adjuvant therapy for cancer, has been shown to induce DIR-1 expression in the early generation of cells, the percentage being 90% [23]. Recently, p62S200 has been shown to bind to the RFP domain of p62 and stimulate its autophagy activity [24].

Given the role of p62 in modulating innate immune responses in infected macrophages, p62 has emerged as a promising target for therapeutic intervention [19]. Recent studies show that DIR-1 or p62S200, induced based on natural microvesicle particles, regulates heat response, prevents tissue damage and increases host defense following infection [20–22]. Moreover, immunotherapy, an adjuvant therapy for cancer, has been shown to induce DIR-1 expression in the early generation of cells, the percentage being 90% [23]. Recently, p62S200 has been shown to bind to the RFP domain of p62 and stimulate its autophagy activity [24].

High efficacy of dasquetide and its capability to prime the immune system against tumor cells have been demonstrated in animal models of disease. The molecular details underlying its activity remain unidentified. Here, we report the

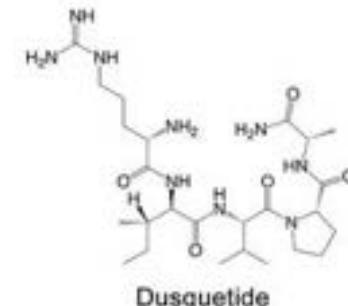
Structure 18, 1–11, August 4, 2010 © 2010 Elsevier Ltd. 4



X-ray structure of dusquetide in complex with p62₇₇

PDB code: 7R1Q

Zhang et al., 2022, Structure, 30, P1055



- Next-generation IDR dusquetide penetrates the cell membrane
 - Dusquetide targets the ZZ domain of p62
 - Treatment of cells with dusquetide, which mimics arginylated ligands of p62_{ZZ}, leads to stabilization of the p62-RIP1 complex and an increase in p38 phosphorylation and CEBP/B expression



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