BIOGRAPHICAL SKETCH

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NAME: Alon, Assaf

eRA COMMONS USER NAME: assafalon

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE	FIELD OF STUDY
Tel Aviv University, Tel Aviv	BS	08/2005	Biotechnology
Weizmann Institute of Science, Rehovot	PHD	03/2012	Structural Biology
Weizmann Institute of Science, Rehovot	Postdoctoral Fellow	03/2015	Computational biology and protein design
Whitehead Institute, Cambridge, MA	Postdoctoral Fellow	12/2015	Structural Studies of immune receptors
Harvard Medical School, Boston, MA	Postdoctoral Fellow	11/2022	Structural and Pharmacological study of membrane protein receptors

A. Personal statement

Cholesterol is an essential component in mammalian cell membranes, critical to maintain their biophysical properties. Moreover, cholesterol is the precursor to a myriad of important bioactive sterols such as oxysterols, vitamin D, bile acids, and the sex hormones androgen, progesterone, and estrogen. Sterols are sensed by numerous classes of receptors and synthesized by a multitude of enzymes that are tightly regulated. Severe disease results when dysregulation causes sterol accumulation or depletion. In my lab we use a combination of structural biology, pharmacology, biochemistry, computational methods, and cell biology to decipher the molecular mechanisms underlying sterol synthesis, recognition, and signaling.

My graduate work in Deborah Fass' lab at the Weizmann Institute of Science focused on the structure and function of the secreted multidomain disulfide catalyst quiescin sulfhydryl oxidase (QSOX). Using X-ray crystallography, I solved the first structures of QSOX and by various biophysical techniques demonstrated its dynamic nature. Furthermore, I developed tools to inhibit QSOX and probe its role in ECM assembly. In the lab of Sarel Fleishman I developed computational tools for antibody design using the Rosetta software suite. In the lab of Andrew Kruse at Harvard Medical School I studied the sigma-2 receptor (σ_2), an orphan receptor implicated in cancer and neuropsychiatric diseases. Despite being the target of several drugs in clinical trials, the molecular identity of the receptor was unknown until I cloned the σ_2 receptor from tissue and showed that it is TMEM97, a four-helix ER-resident protein. I then used X-ray crystallography to solve structures of σ_2 in complex with several high affinity ligands. These structures show in molecular detail how ligands are recognized. Working with Brian Shoichet and Allan Basbaum at UCSF, I used virtual docking to discover and optimize new σ_2 ligands that were anti-allodynic in a mouse model of neuropathic pain.

1. **Alon A**, Lyu J, Braz JM, Tummino TA, Craik V, O'Meara MJ, Webb CM, Radchenko DS, Moroz YS, Huang XP, Liu Y, Roth BL, Irwin JJ, Basbaum AI, Shoichet BK, Kruse AC. Structures of the σ_2 receptor enable docking for bioactive ligand discovery. ***Nature***. 2021 Dec;600(7890):759-764. PubMed Central PMCID: PMC8867396.
2. **Alon A**, Schmidt HR, Wood MD, Sahn JJ, Martin SF, Kruse AC. Identification of the gene that codes for the σ_2 receptor. ***Proc Natl Acad Sci U S A***. 2017 Jul 3;114(27):7160-7165. PubMed Central PMCID: PMC5502638.
3. Ilani T, **Alon A**, Grossman I, Horowitz B, Kartvelishvily E, Cohen SR, Fass D. A secreted disulfide catalyst controls extracellular matrix composition and function. ***Science***. 2013 Jul 5;341(6141):74-6. PubMed PMID: 23704371.

4. **Alon A**, Grossman I, Gat Y, Kodali VK, DiMaio F, Mehlman T, Haran G, Baker D, Thorpe C, Fass D. The dynamic disulphide relay of quiescin sulphydryl oxidase. ***Nature***. 2012 Aug 16;488(7411):414-8. PubMed Central PMCID: PMC3521037.

B. Positions, scientific appointments and honors

Positions and scientific appointments

2007 – 2012	Graduate Research, Weizmann Institute of Science, Rehovot
2013 – 2015	Postdoctoral Fellow, Weizmann Institute of Science, Rehovot
2016 – 2022	Postdoctoral Fellow, Harvard Medical School, Boston, MA
December 2022	Assistant Professor, Yale University School of Medicine, New Haven, CT

Honors

2017	The Merck Postdoctoral Fellowship, Merck
2016	Outstanding Postdoc Fellow Award, Harvard Medical School
2013	Biochemistry Dean postdoctoral award, Weizmann Institute of Science
2005	Dean's honor list, Tel Aviv University
2003	Dean's honor list, Tel Aviv University

Professional societies and public advisory committees:

2013 – 2015:	Member – RosettaCommons
2014 – present:	Member – The Protein Society
2016	Reviewer – Protein Science
2017	Reviewer – Scientific reports
2020	Reviewer – Communications Biology

Teaching and mentoring experience:

My teaching experience comes from my army service as a medical instructor and from serving as TA on various courses at the Weizmann Institute of Science, most notable for the classes "Chemistry of Life" and "Basic Techniques in Protein Biochemistry".
My mentoring experience comes from mentoring undergraduate students, graduate students, and postdoctoral fellows in the lab, both during my graduate studies and my postdoctoral research. I supervised experiments, instructed on various techniques and instruments, helped with preparing presentations and manuscripts, and provided feedback and advice on interviewing for fellowships and academia and industry positions.

C. Contribution to science

1. Postgraduate work:

The σ_2 receptor – from a pharmacological mystery to structure- based drug discovery

In the lab of Andrew Kruse at Harvard Medical School I studied the sigma-2 receptor (σ_2), an orphan receptor implicated in cancer, pain, and neuropsychiatric diseases such as Alzheimer's disease and schizophrenia. The σ family was discovered in 1976 and subdivided in 1990 into the two σ "subtypes", σ_1 and σ_2, that share overlapping pharmacology but differ in tissue distribution and molecular weight. The gene for σ_1 was identified in 1996, and no homologs were identified in the human genome. Mouse knockout studies showed that σ_2 is not a splice variant and is genetically unrelated to σ_1 (despite the name). The molecular identity of σ_2 remained elusive for decades, precluding the use of all modern molecular biology tools to investigate its function. I cloned the σ_2 receptor from calf liver tissue using classical biochemical fractionation techniques. I solubilized liver membranes in mild detergent, followed σ_2 by radioligand binding, and identified candidates by mass spectrometry. I showed that the σ_2 receptor, which had previously been defined purely based on its ligand binding properties, is TMEM97, a four-helix ER-resident protein. I used siRNA knockdown to show that TMEM97 is necessary for σ_2 activity. I

expressed TMEM97 in a system that lacks endogenous TMEM97 (insect cells) and by saturation radioligand binding showed that TMEM97 is sufficient for σ_2 activity. As a next step I wanted to understand what governs σ_2 ligand recognition at the molecular level and how two unrelated proteins with very different folds can have such an overlap in their pharmacology. Membrane proteins are notoriously challenging to study, however recent advances in detergent chemistry and other extraction techniques together with recent developments in lipidic cubic phase crystallography allowed me to purify and crystallize the σ_2 receptor. I solved structures of the σ_2 receptor in complex with cholesterol, the schizophrenia drug candidate roluperidone, and the tool compound PB28. These structures illuminated how ligands are recognized in molecular detail. Although the two σ receptors adopt very different overall folds – σ_1 is a β-barrel cupin fold while σ_2 is a four-helix bundle, placement of similar amino acids in cognate positions in the binding sites explains the overlapping pharmacology of the σ family. The paucity of σ_2-selective ligands has made divorcing the pharmacological response of σ_1 from σ_2 extremely challenging. Working with Jiankun Lyu, a postdoc in the lab of Brian Shoichet, I used virtual docking to computationally screen half a billion small molecules. I used radioligand binding to test and optimize top ranking hits from this screen. Crystal structures of σ_2 in complex with two of these hits demonstrated that the docked pose matched the crystal pose. Since the σ_2 receptor is involved in pain I reached out to Allan Basbaum at UCSF to test our compounds in vivo. We first measured the plasma and CNS concentration and the half- life of the compounds showing substantial brain permeability. We then showed that these novel σ_2 ligands are anti-allodynic in a mouse spare nerve injury (SNI) model of neuropathic pain.

1. **Alon A**, Lyu J, Braz JM, Tummino TA, Craik V, O'Meara MJ, Webb CM, Radchenko DS, Moroz YS, Huang XP, Liu Y, Roth BL, Irwin JJ, Basbaum AI, Shoichet BK, Kruse AC. Structures of the σ_2 receptor enable docking for bioactive ligand discovery. ***Nature***. 2021 Dec;600(7890):759-764. PubMed Central PMCID: PMC8867396.
2. **Alon A**, Schmidt HR, Wood MD, Sahn JJ, Martin SF, Kruse AC. Identification of the gene that codes for the σ_2 receptor. ***Proc Natl Acad Sci U S A***. 2017 Jul 3;114(27):7160-7165. PubMed Central PMCID: PMC5502638.
3. **Alon A**, Schmidt H, Zheng S, Kruse AC. Structural Perspectives on Sigma-1 Receptor Function. ***Adv Exp Med Biol***. 2017;964:5-13. PubMed PMID: 28315261.

2. Postgraduate work:

Using computational protein design to switch antibody species preference

I joined the lab of Sarel Fleishman, also at the Weizmann, and developed computational methods for antibody design. The inhibitory antibody that I isolated from mice did not bind the mouse QSOX owing to self- tolerance, precluding the use of this antibody in mouse models of cancer. I developed computational methods to switch species preference of antibodies. I screened my computationally designed antibodies and crystallized two high affinity binders. These structures shed light on shortcomings in the algorithm and allowed me to suggest improvements. In addition, I used these computational tools to come up with a general method to humanize antibodies from different species.

1. Lapidoth GD, Baran D, Pszolla GM, Norn C, **Alon A**, Tyka MD, Fleishman SJ. AbDesign: An algorithm for combinatorial backbone design guided by natural conformations and sequences. ***Proteins***. 2015 Aug;83(8):1385-406. PubMed Central PMCID: PMC4881815.

3. Graduate work:

Structure, dynamics, and inhibition of a secreted disulfide catalyst

My graduate work in Deborah Fass' lab at the Weizmann Institute of Science focused on the structure and function of the disulfide catalyst quiescin sulfhydryl oxidase (QSOX). Disulfide bonds are typically catalyzed in the endoplasmic reticulum (ER) by two proteins, PDI and EroI, working sequentially. PDI, an oxidoreductase, exchanges its active site disulfide with two cysteines in a client protein to form a new

disulfide bond. PDI then gets recycled by EroI, a sulfhydryl oxidase that can generate disulfides de novo by transferring electrons to molecular oxygen, the terminal electron acceptor. QSOX is unique in two ways. First, unlike all other disulfide catalysts, it is made from two modules, an oxidoreductase, and a sulfhydryl oxidase, which allows it to catalyze disulfide bonds in client proteins without the need of an additional partner. Second, QSOX is the only disulfide catalyst that operates downstream of the ER. QSOX is either located to the Golgi apparatus or secreted to the extracellular matrix (ECM). How the two modules communicate to transfer the electrons from one module to the other was unclear. I solved the crystal structure of QSOX in a resting configuration, “waiting for substrate”. Using mutagenesis together with creative purification techniques I was able to trap QSOX in an intermediate conformation with a mixed disulfide, formed by the nucleophilic attack of a cysteine in one module on the disulfide of the other module. I combined insights from these structures, data from FRET experiments, and cross-linking mass spectrometry (XL-MS) experiments to show that the two modules of QSOX are linked together by a flexible tethered and that QSOX samples many conformations in solution. QSOX is produced by fibroblasts as they enter quiescence and is over-expressed in prostate cancers and in pancreatic adenocarcinoma. Because QSOX is secreted to the ECM we hypothesized that its enzymatic activity could influence metastatic cancer. Along with structural and enzymatic insights, I made finding a means to inhibit QSOX one of my primary research goals. I immunized mice with QSOX and generated a library of monoclonal antibodies, which I then screened for monoclonal antibodies that could both bind and inhibit the enzymatic activity of QSOX. I identified a powerful clone that could bind QSOX with 4 nM affinity and completely block catalytic activity. This clone was further characterized and developed in the lab. Using this inhibitory antibody, I also studied the role QSOX plays in laminin ECM incorporation and cell migration.

1. Grossman I, **Alon A**, Ilani T, Fass D. An inhibitory antibody blocks the first step in the dithiol/disulfide relay mechanism of the enzyme QSOX1. ***J Mol Biol***. 2013 Nov 15;425(22):4366-78. PubMed PMID: 23867277.
2. Ilani T, **Alon A**, Grossman I, Horowitz B, Kartvelishvily E, Cohen SR, Fass D. A secreted disulfide catalyst controls extracellular matrix composition and function. ***Science***. 2013 Jul 5;341(6141):74-6. PubMed PMID: 23704371.
3. **Alon A**, Grossman I, Gat Y, Kodali VK, DiMaio F, Mehlman T, Haran G, Baker D, Thorpe C, Fass D. The dynamic disulphide relay of quiescin sulphydryl oxidase. ***Nature***. 2012 Aug 16;488(7411):414-8. PubMed Central PMCID: PMC3521037.
4. **Alon A**, Heckler EJ, Thorpe C, Fass D. QSOX contains a pseudo-dimer of functional and degenerate sulfhydryl oxidase domains. ***FEBS Lett***. 2010 Apr 16;584(8):1521-5. PubMed PMID: 20211621.

Complete List of Published Work in My Bibliography:
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