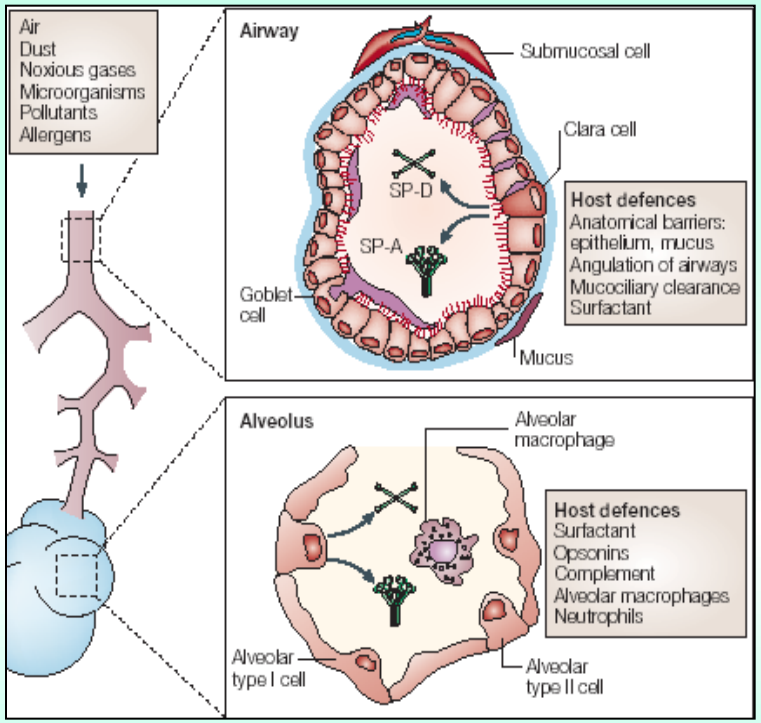
Alterations in Multimeric Structure of Surfactant Protein D as a Biomarker for Lung Injury and Inflammation in Humans

Elena Atochina-Vasserman, Ph.D., M.D.

University of Pennsylvania

School of Medicine

Surfactant Protein D



• Surfactant protein D is a Ca^{2+} -binding member of Collagen-like lectins ("collectins") that play a role in non-antibody-mediated innate immune responses.

• SP-D shares considerable structural homology with other proteins of this type (including SP-A, conglutinin, bovine collectin-43, and mannose binding protein).

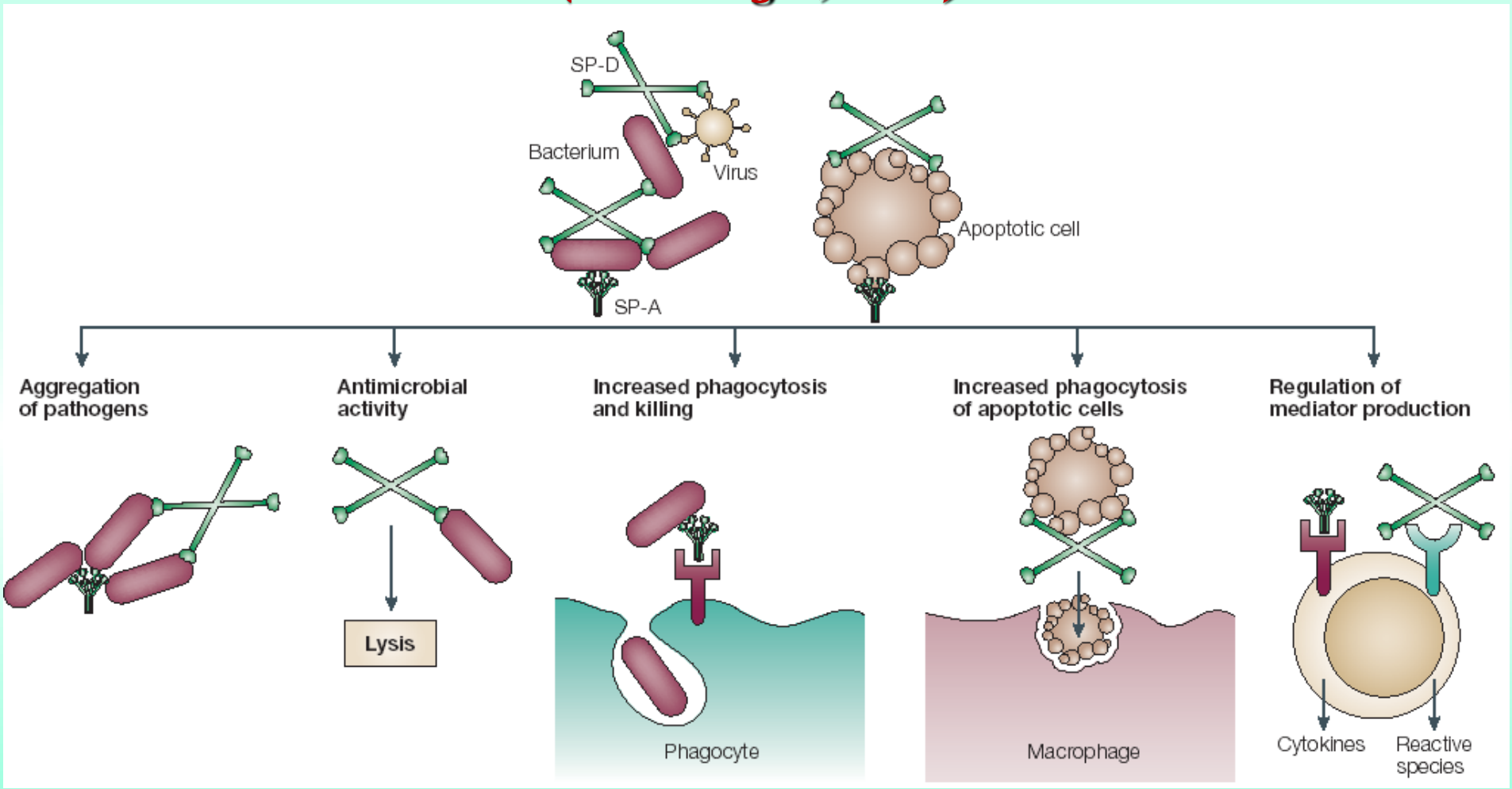
• SP-D is produced primarily by alveolar type II cells and nonciliated bronchiolar cells in the lung.

• The primary function of the pulmonary collectins appears to be the modulation of host defense and inflammation.

JR Wright, 2004

Function of SP-A and SP-D

(J.R. Wright, 2006)

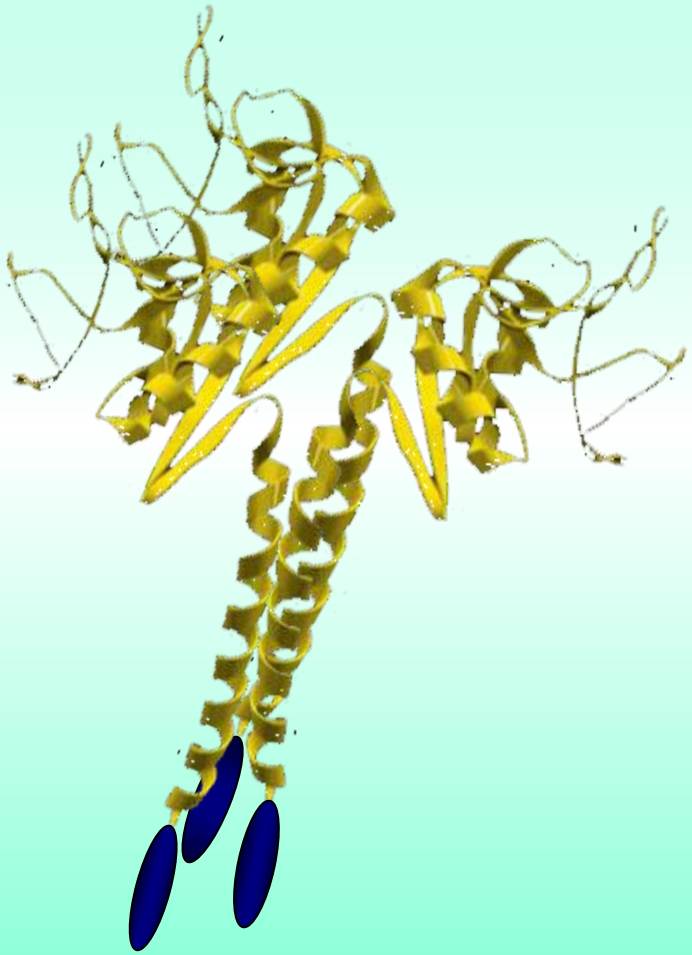


SP-A and SP-D bind to a variety of bacteria, viruses, allergens and apoptotic cells and thereby function as opsonins to enhance the uptake of these cells and particles. Binding of the collectins to pathogens occurs by various mechanisms.

SP-A and SP-D also have direct effects on immune cells and modulate the production of cytokines and inflammatory mediators.

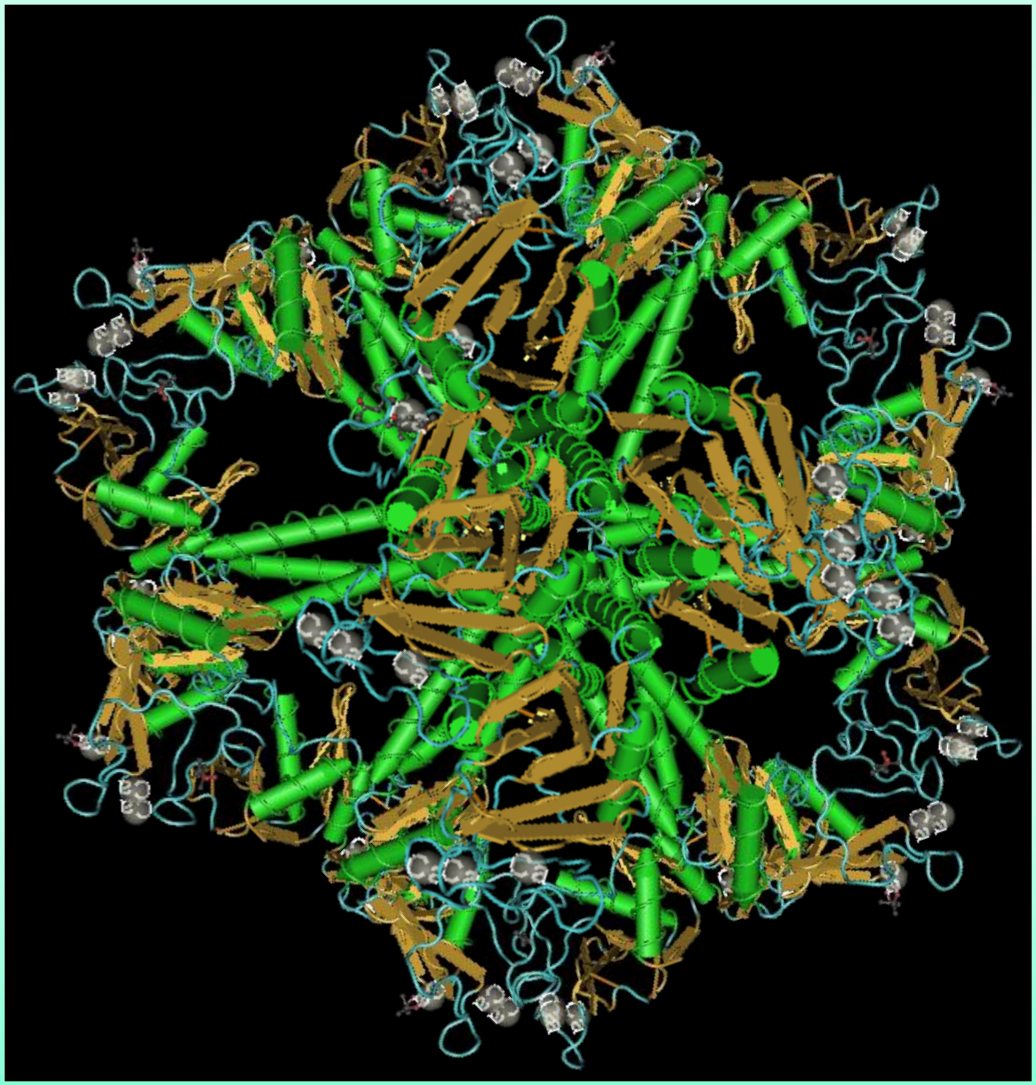
Collectin monomers are highly oligomerized

The head and neck domains drive the aggregation of the SP-D monomer to form a trimer of ~130 kDa



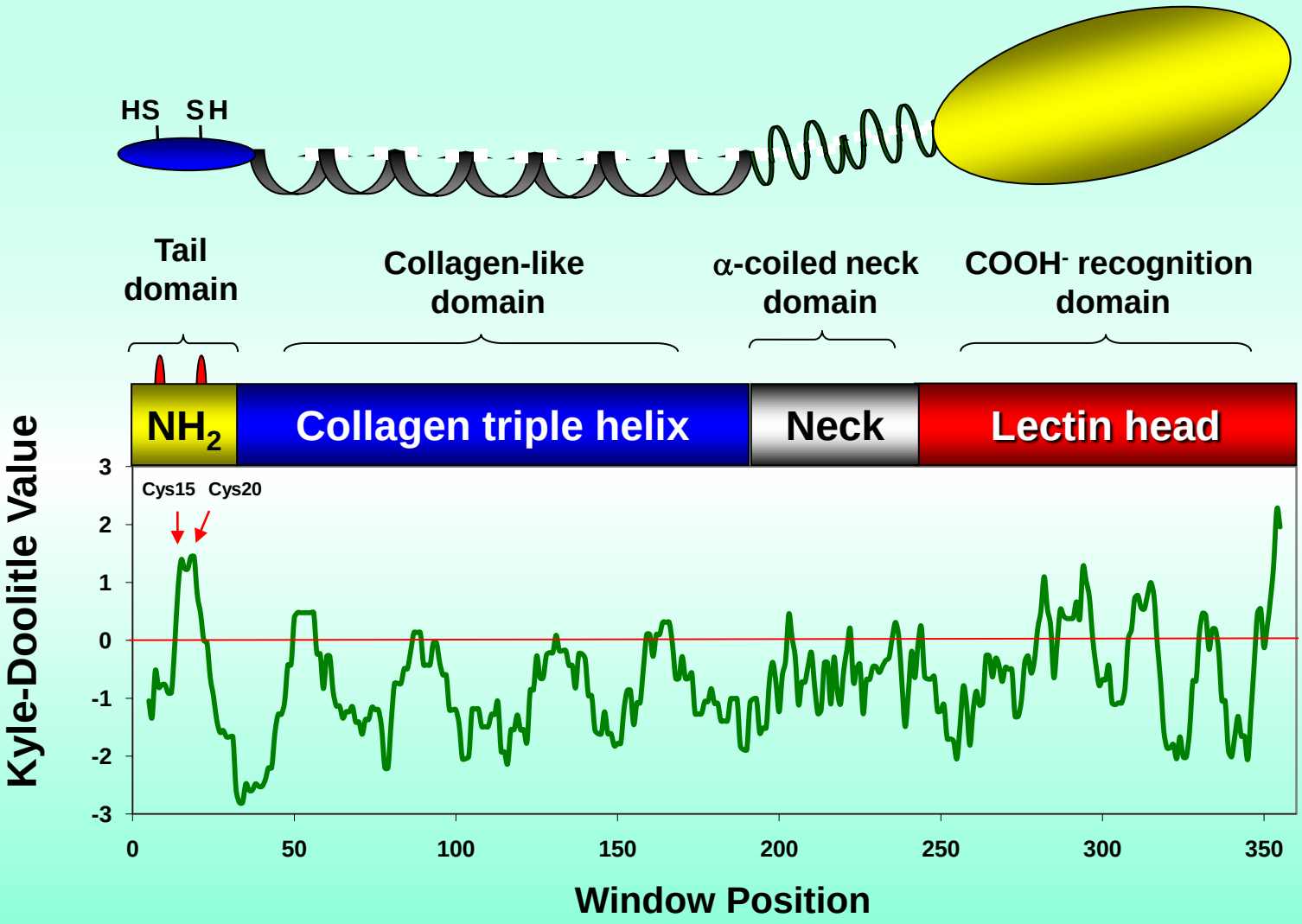
SP-D can form multimers, so-called “fuzzy balls” with a total mass of several million kDa and a size of about 100 nm

Oligomerization of SP-D results in the masking of the tail domains while the head domains remains exposed



size of about 100 nm

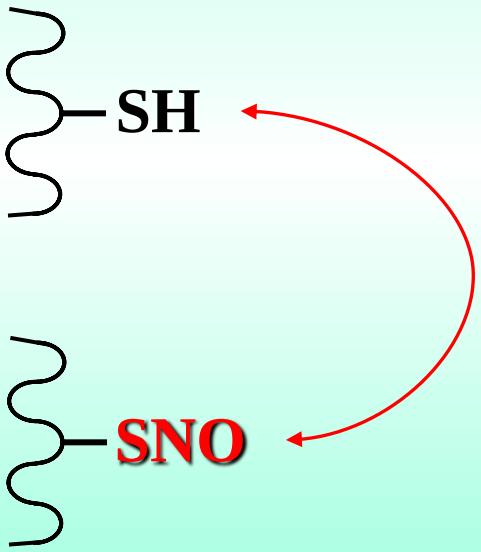
Monomeric Structure of SP-D



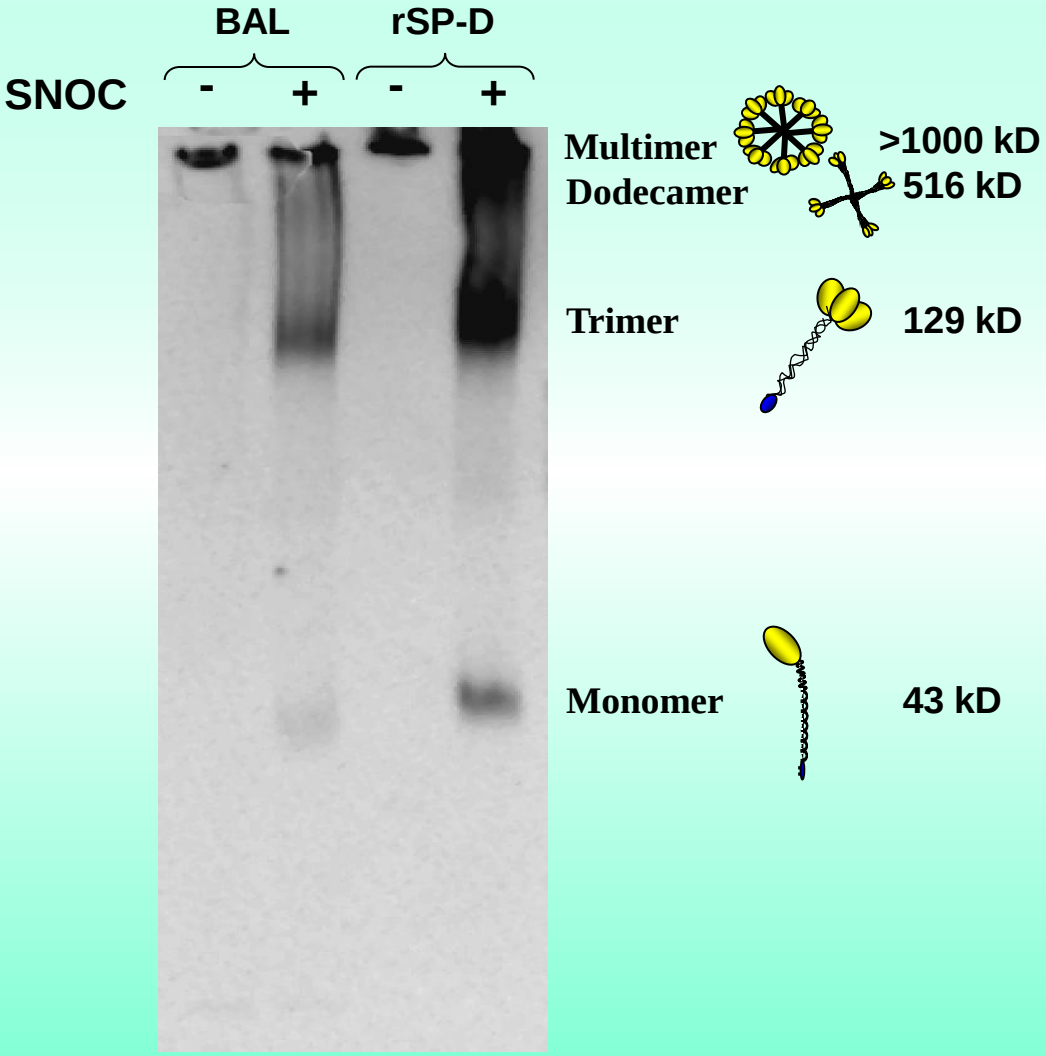
There are 6 cysteines within SP-D that potentially could be nitrosylated (2 in the NH2-terminal region and 4 in the carbohydrate- binding domain). However, only two cysteines 15 and 20 are situated within the hydrophobic tail region, as a motif for nitrosylation.

SP-D multimeric structure can be altered by *S*-nitrosylation *in vitro*

S-nitrosylation is a covalent modification of thiol groups resulting in S-nitrosothiol (SNO) formation.

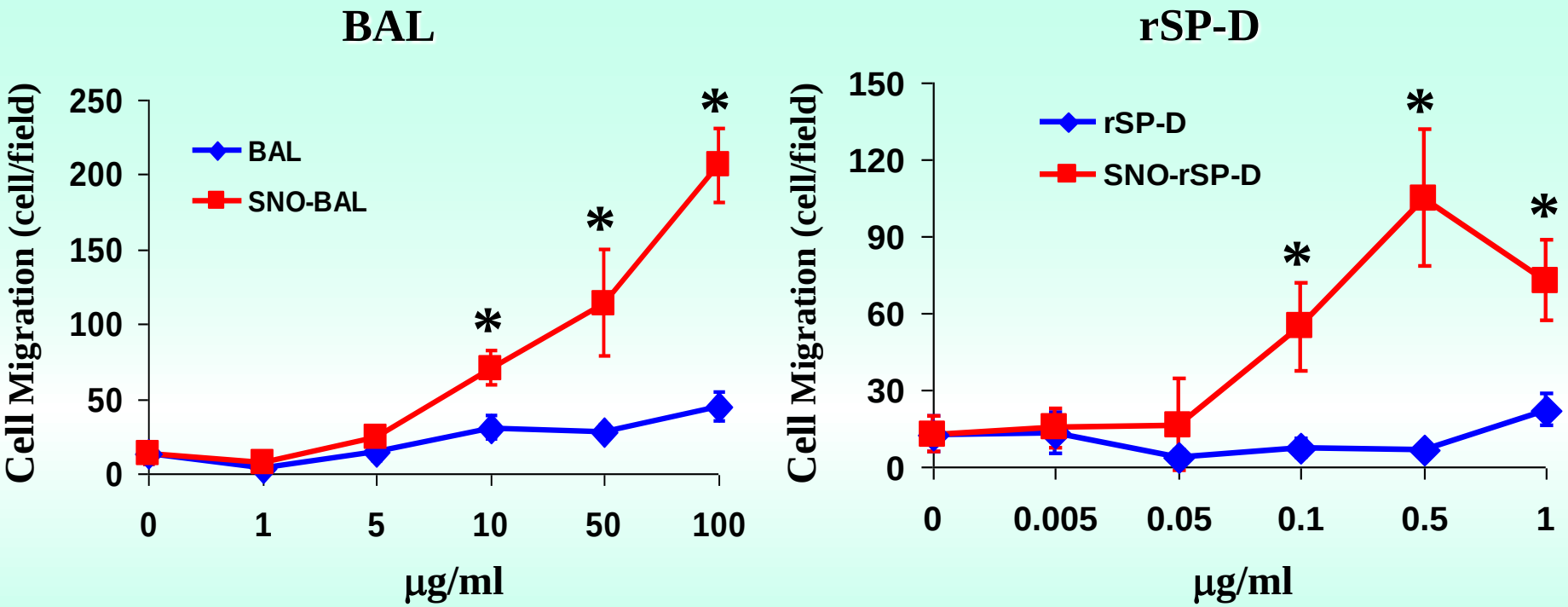


SNOC (*S*-nitrosocysteine)



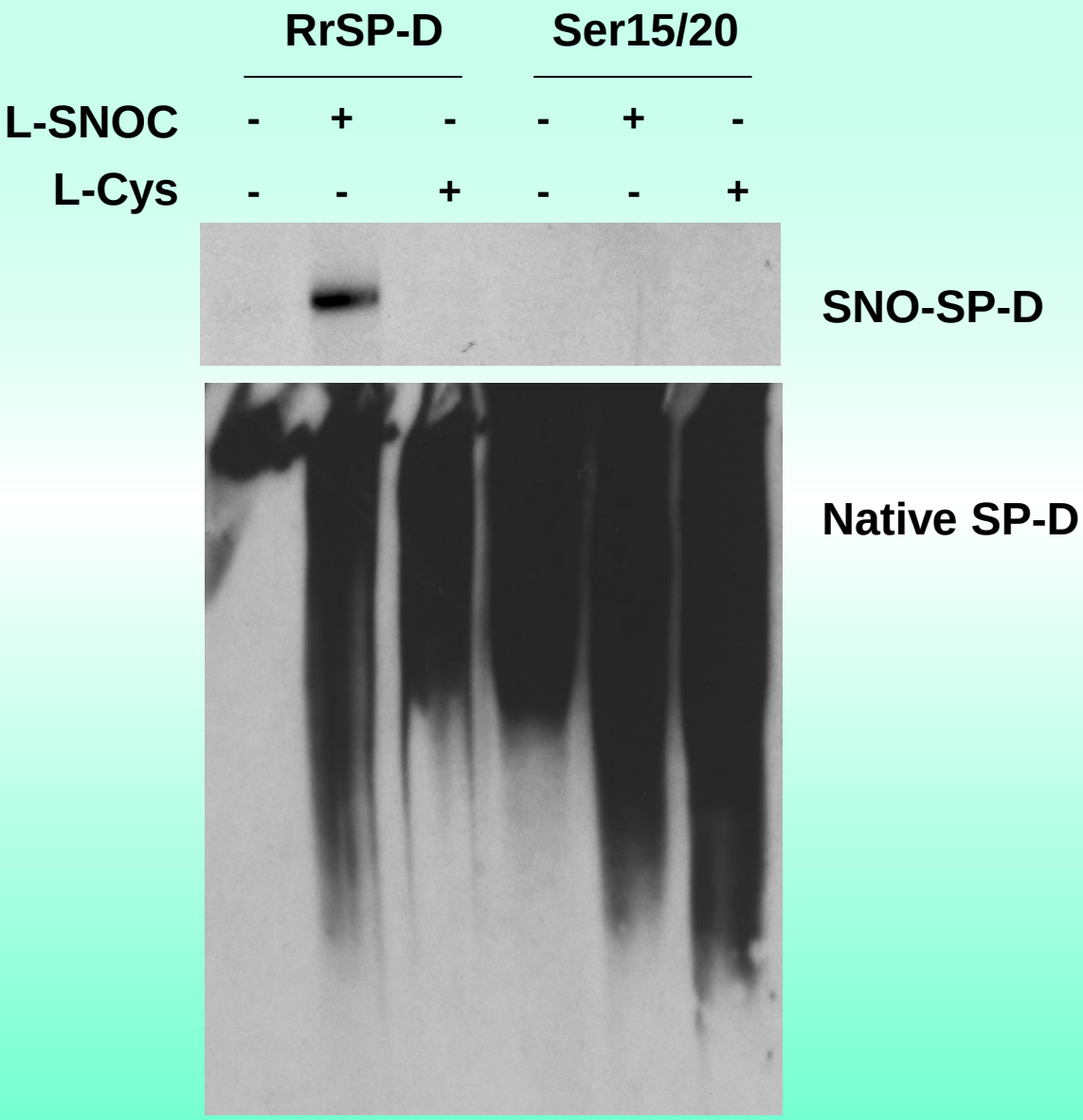
CJ Guo, E Atochina-Vasserman et al, *PLoS Biology*, 2008

S-nitrosylation of normal BAL and rSP-D induces macrophage chemotactic function

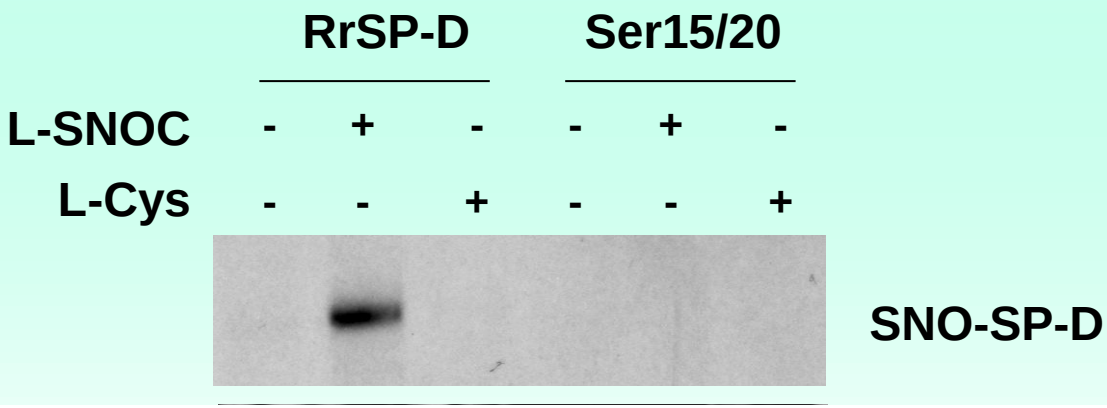


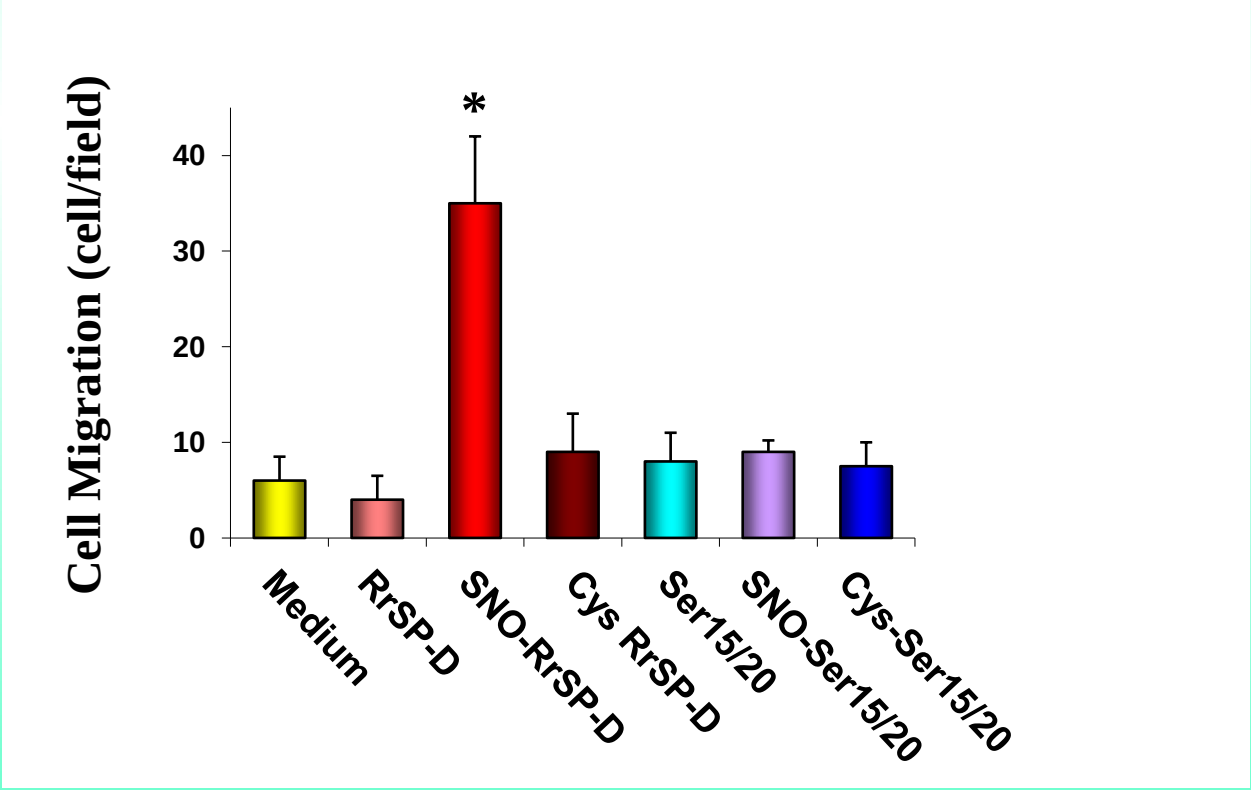
- S-nitrosylation unmasks a pro-inflammatory function of SP-D
- SNO-SPD is a chemoattractant

Signaling in SP-D is NO-Dependent

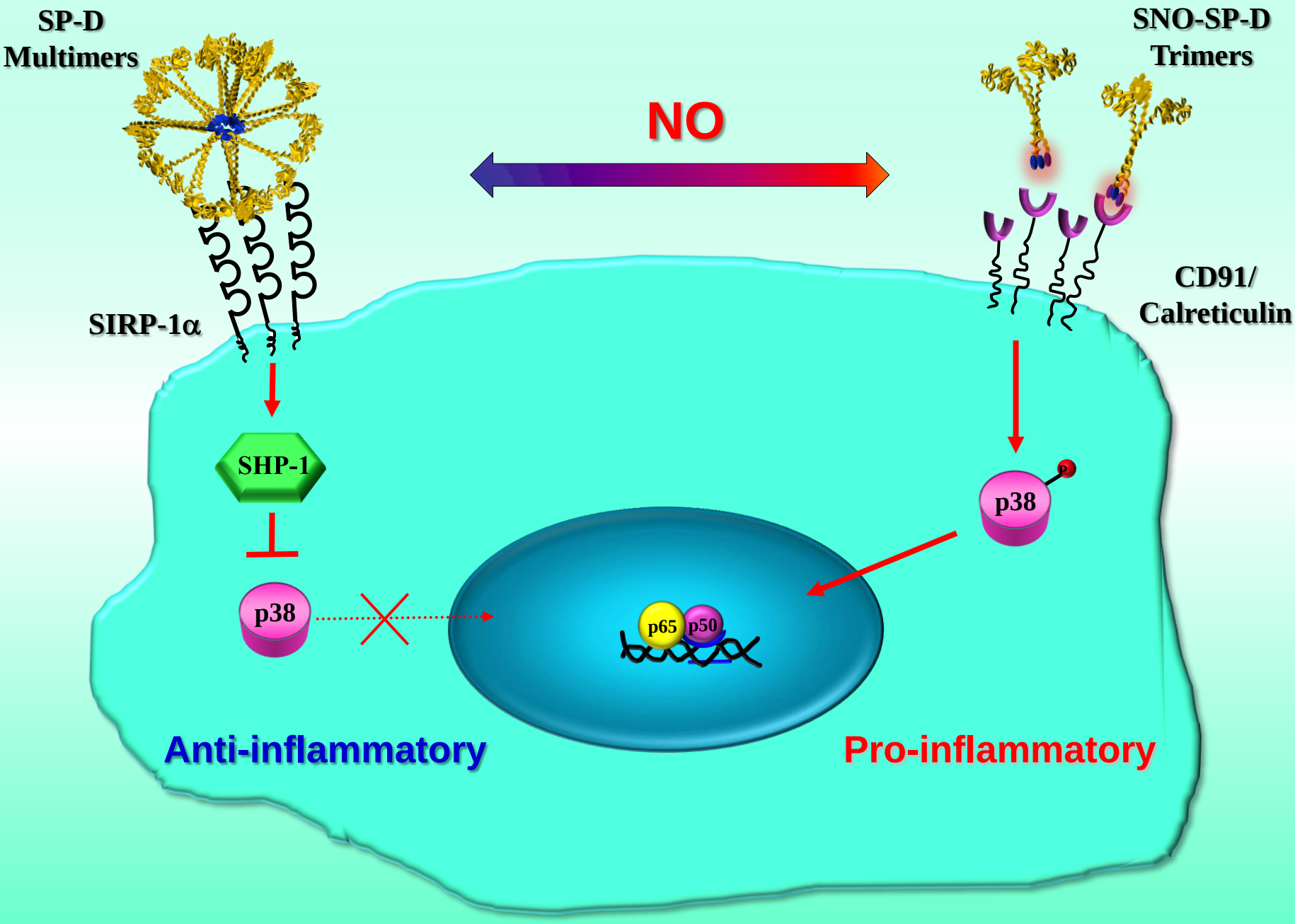


Signaling in SP-D is NO-Dependent

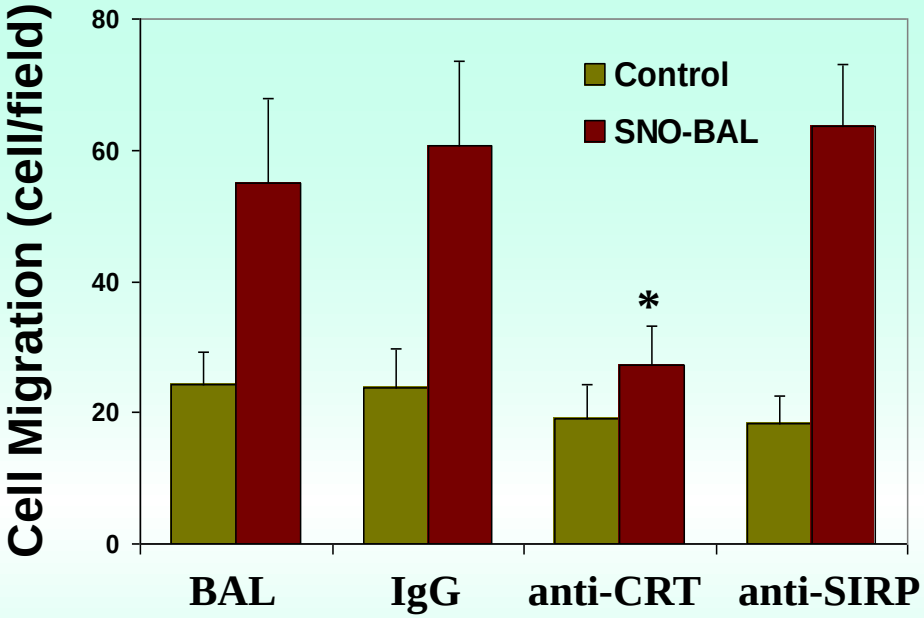


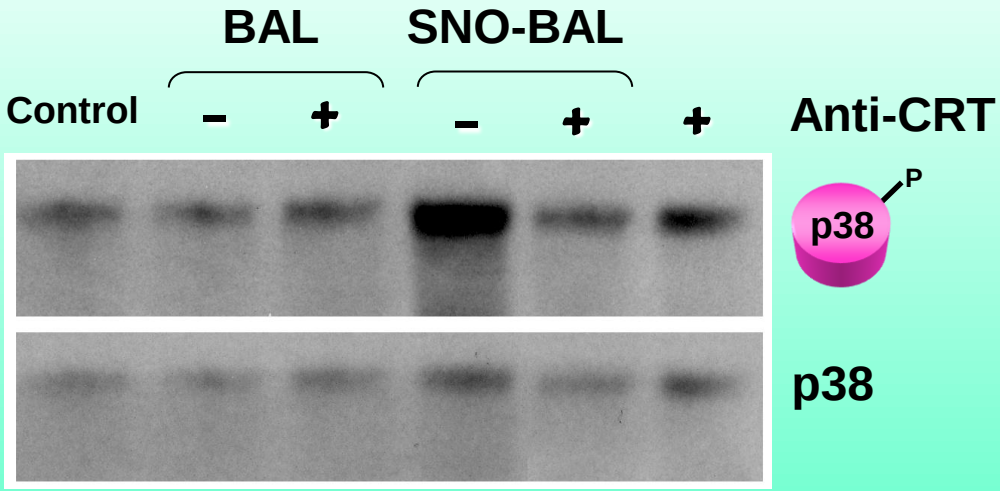


Model of the pro and anti-inflammatory functions of SP-D



Pretreatment with anti-CRT antibody specifically reduced the SNO-BAL mediated chemotaxis and p38 phosphorylation

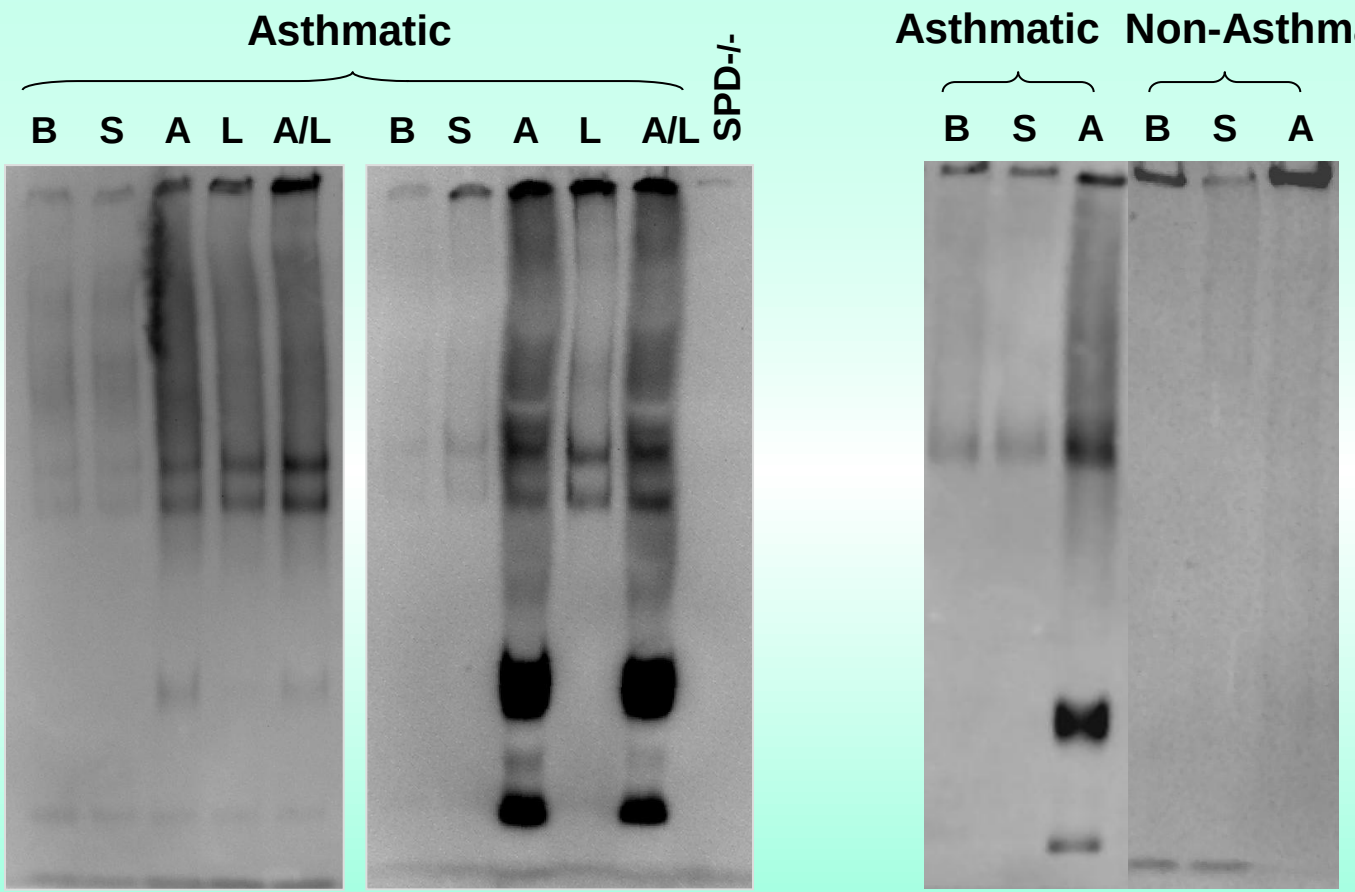




The human inflammatory lung diseases, Asthma and Hermansky Pudlak Syndrome type 1 (HPS1) are associated with increases of SP-D in BAL and amplification of inflammatory signals in the distal lung

We hypothesize that post-translational modification of SP-D with disruption of its multimeric structure is associated with inflammatory lung disease in humans

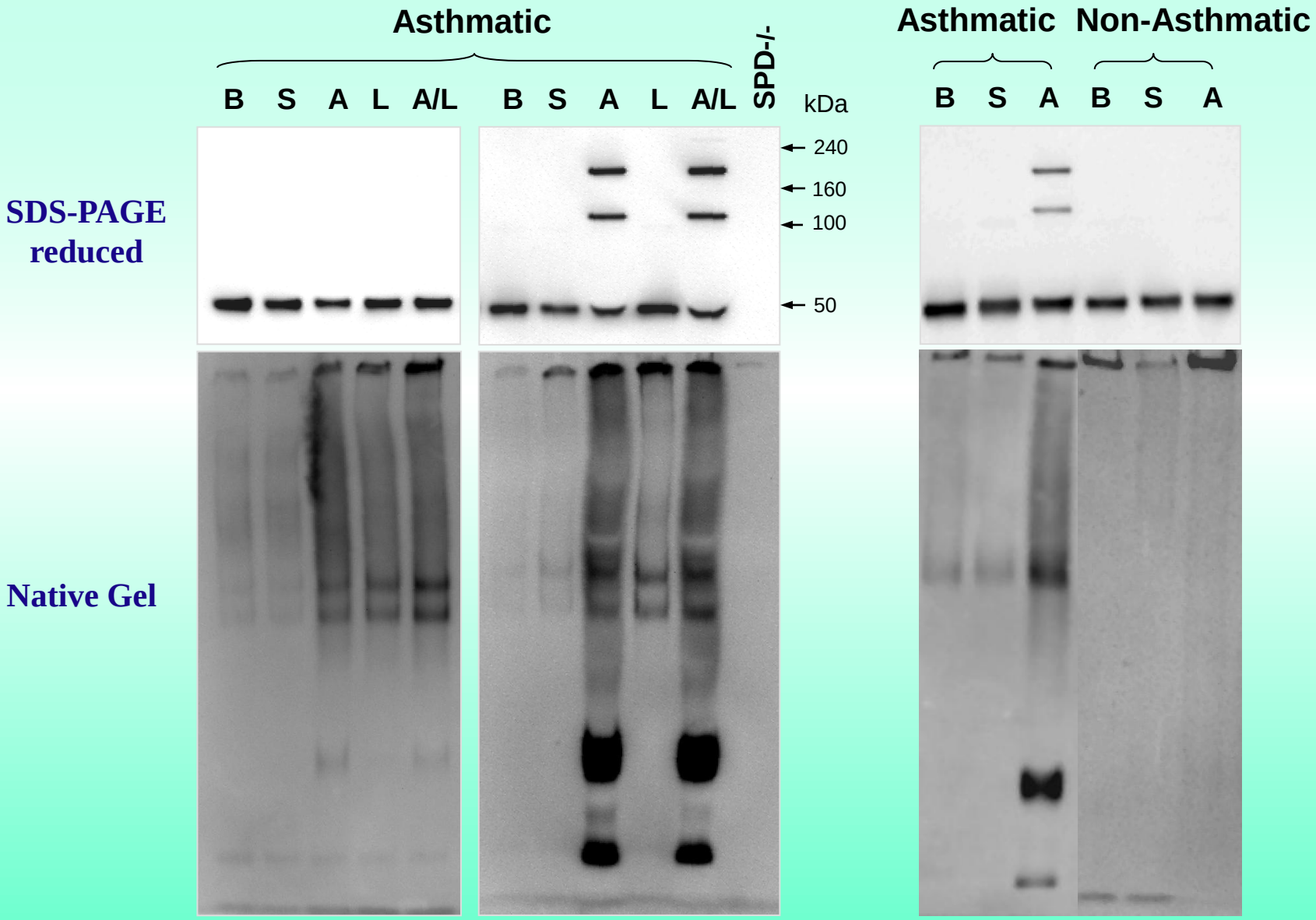
Multimeric structure of SP-D is altered after segmental challenge



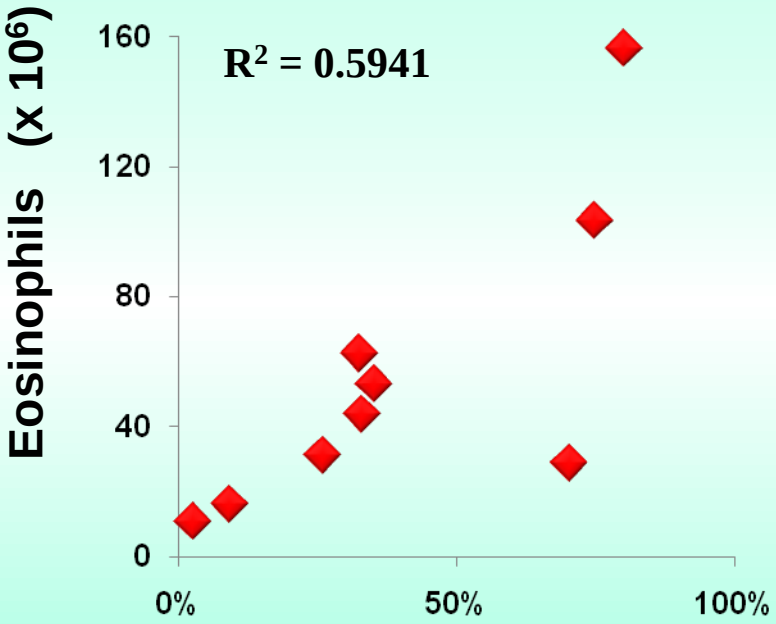
Non-asthmatic patients did not develop cross-linked SP-D bands and SP-D multimeric structure was not disrupted after the same procedure

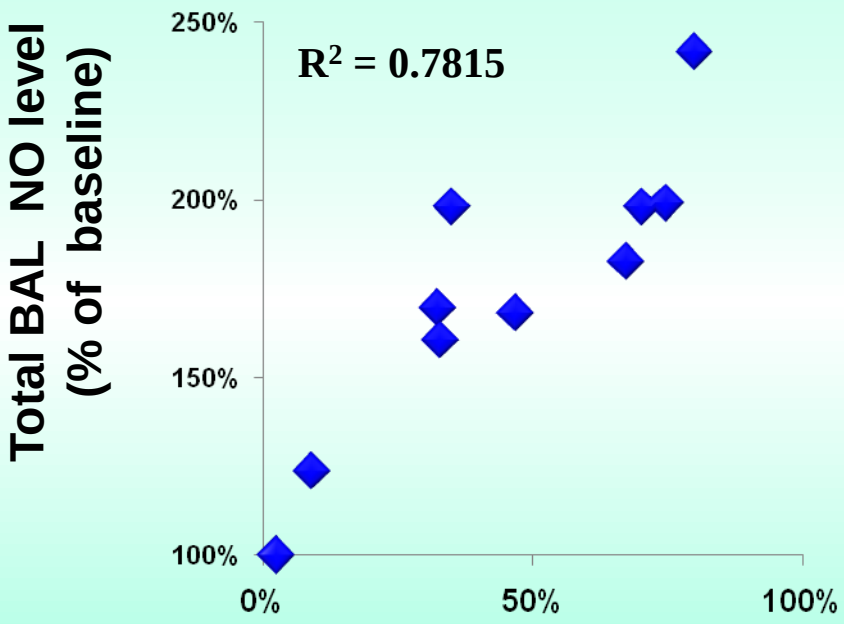
E Atochina-Vasserman et al, *AJRCCM*, 2010

8 out of 16 asthmatic patients formed cross-linked SP-D after segmental challenge



Cross-linked SP-D is correlated with increased BAL eosinophilia and increased NO levels after segmental challenge



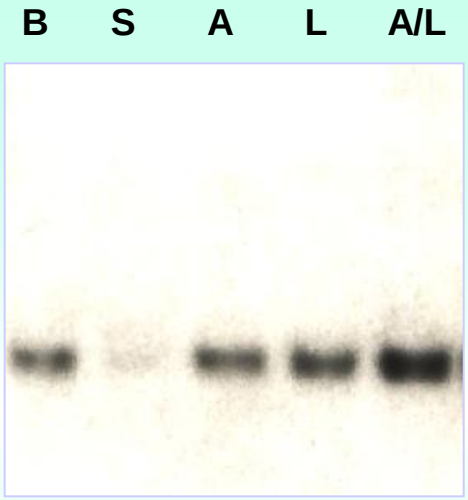


Cross-linked SP-D (% of total unmodified SP-D)

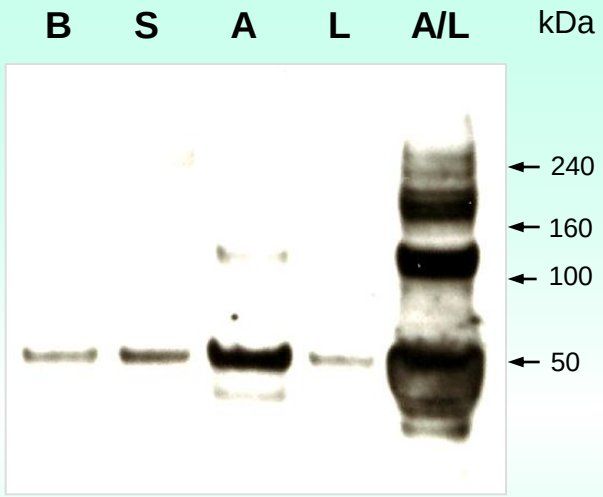
E Atochina-Vasserman et al, *AJRCCM*, 2010

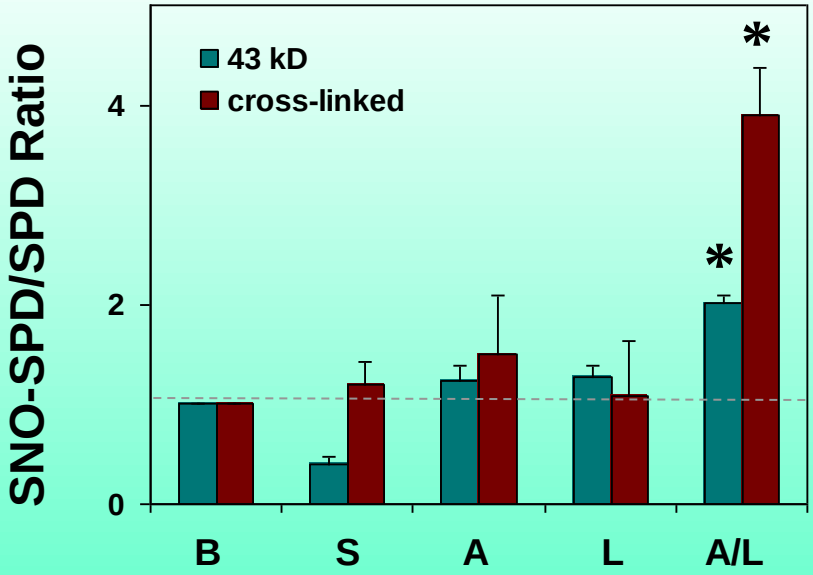
Cross-linked SP-D is associated with SNO-SPD formation after segmental challenge

Patients with monomeric SP-D

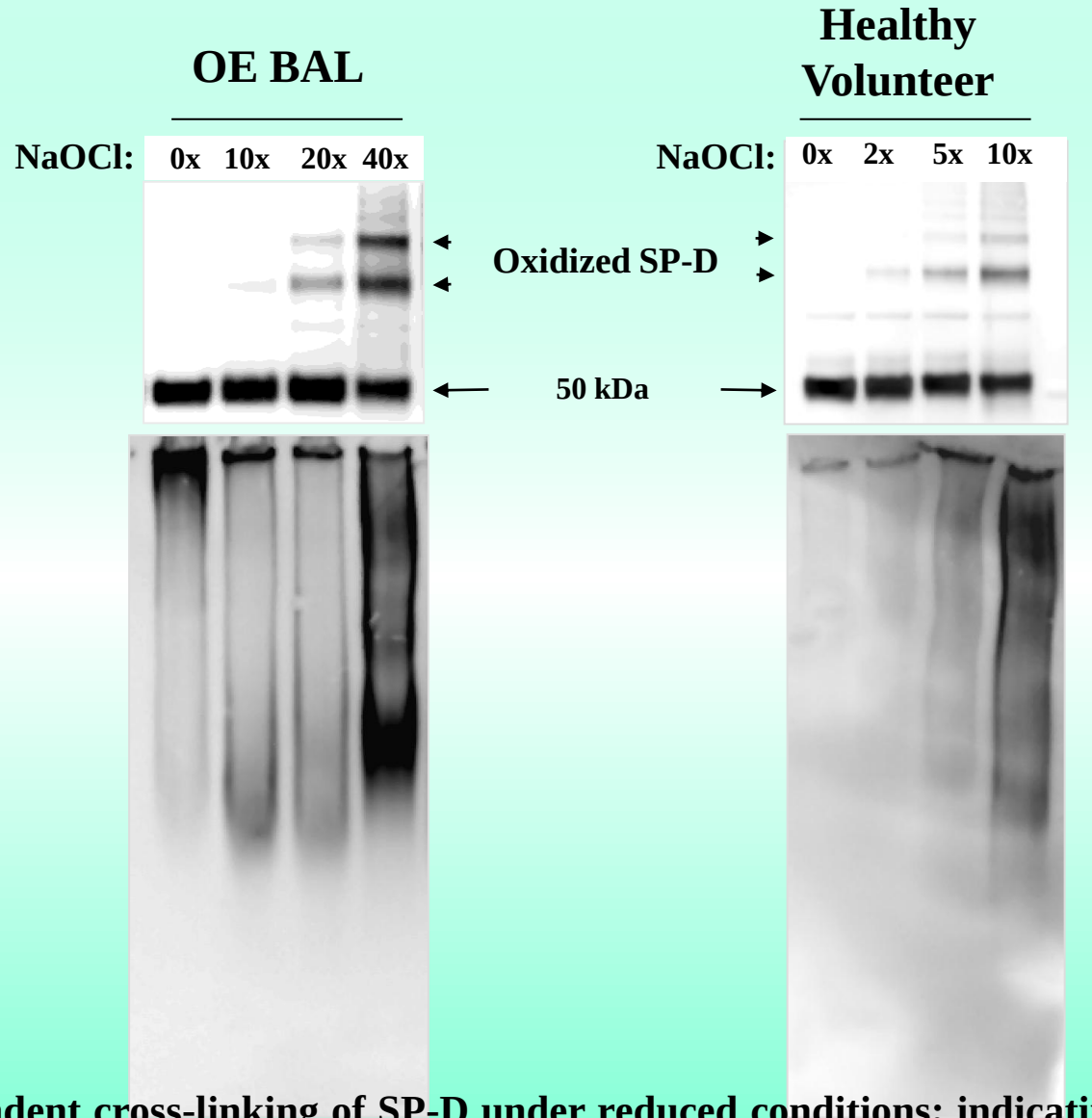


Patients with cross-linked SP-D



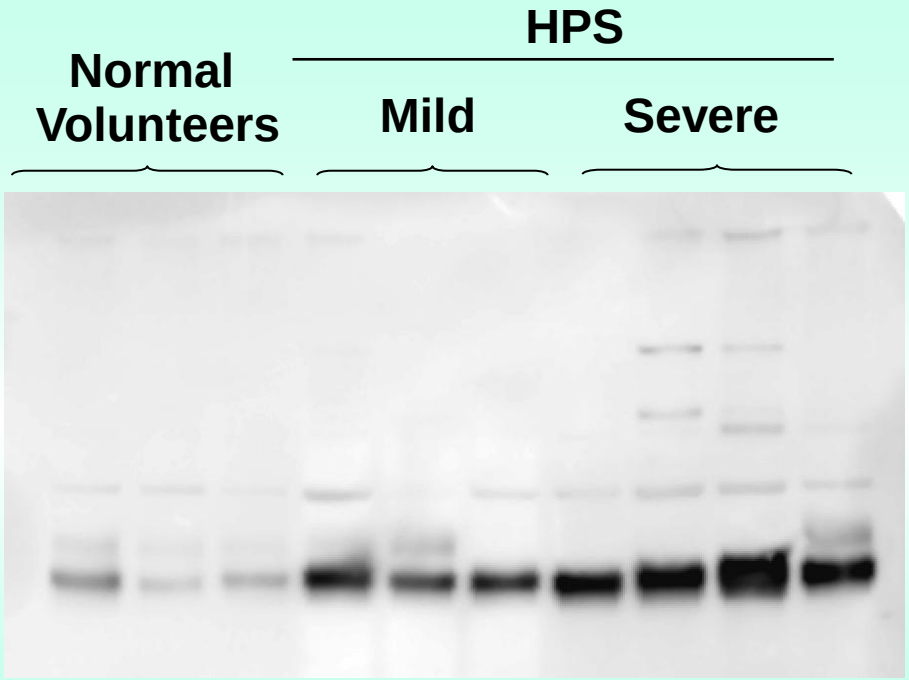


Oxidative cross-linking of SP-D *in vitro*



Dose-dependent cross-linking of SP-D under reduced conditions; indicating the formation of non-disulfide covalent cross-linked forms of SP-D similar to those that appear in the BAL of asthmatic patients after segmental challenged

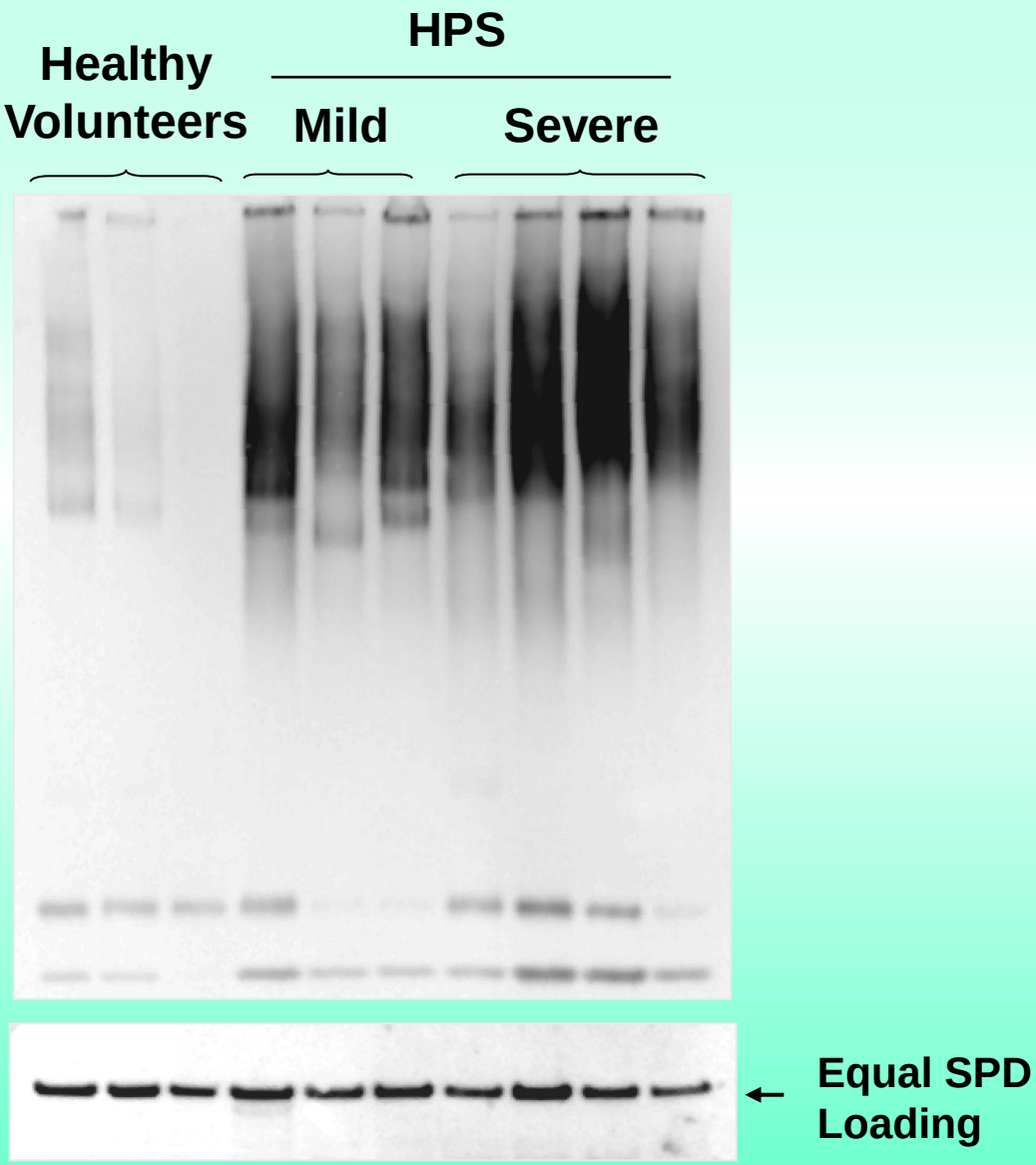
BAL SP-D content increases with increasing disease severity in HPS1 patients



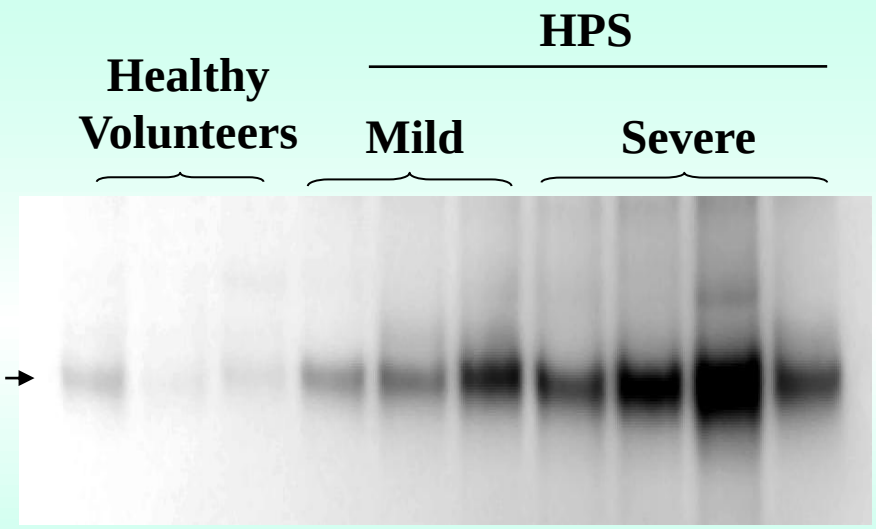
Representative immunoblot for total SP-D on samples of BAL from normal volunteers and HPS1 patients

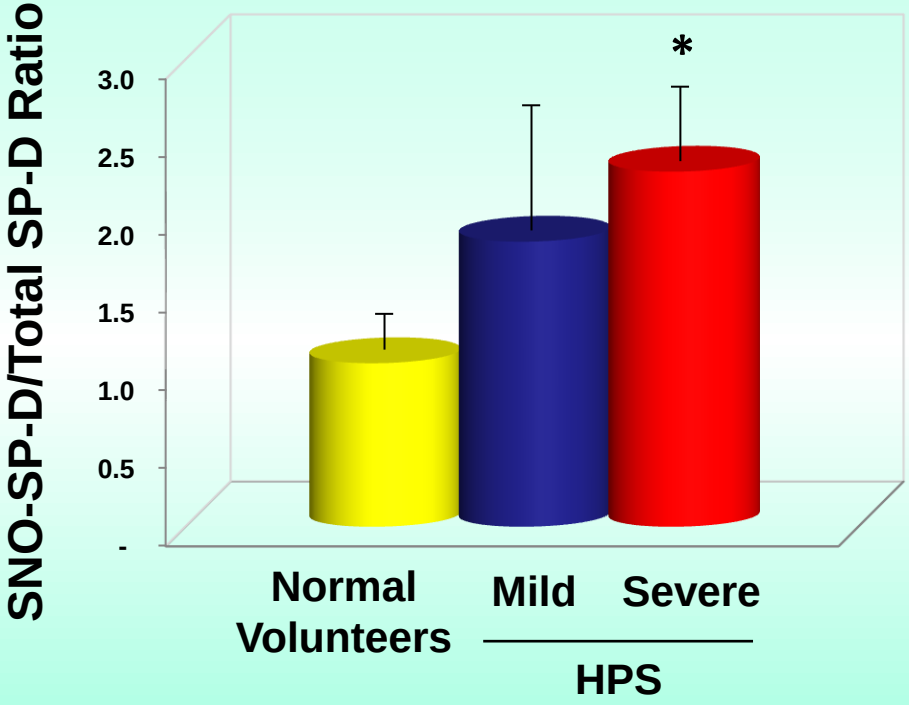
E Atochina-Vasserman et al, JCI, submitted, 2010

SP-D structural disruption is associated with pulmonary inflammation in HPS



SNO-SP-D is associated with pulmonary inflammation in HPS

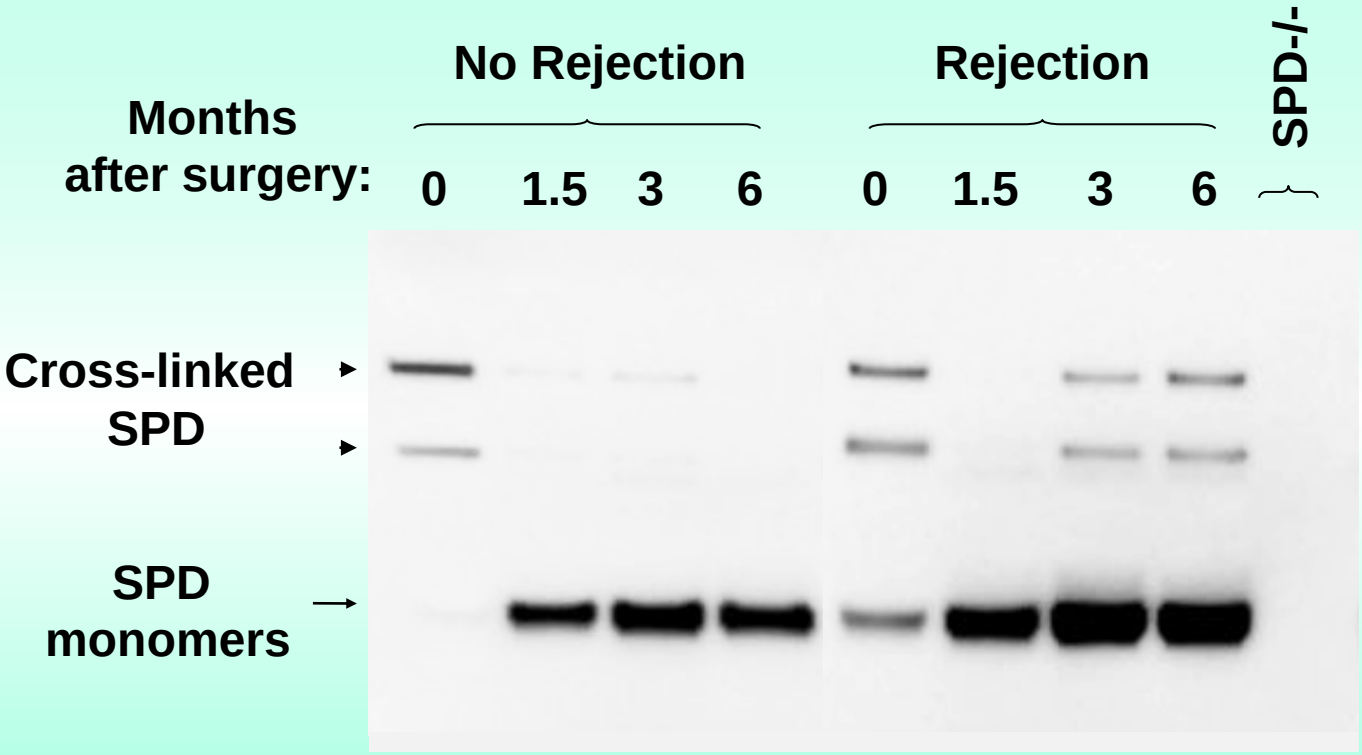




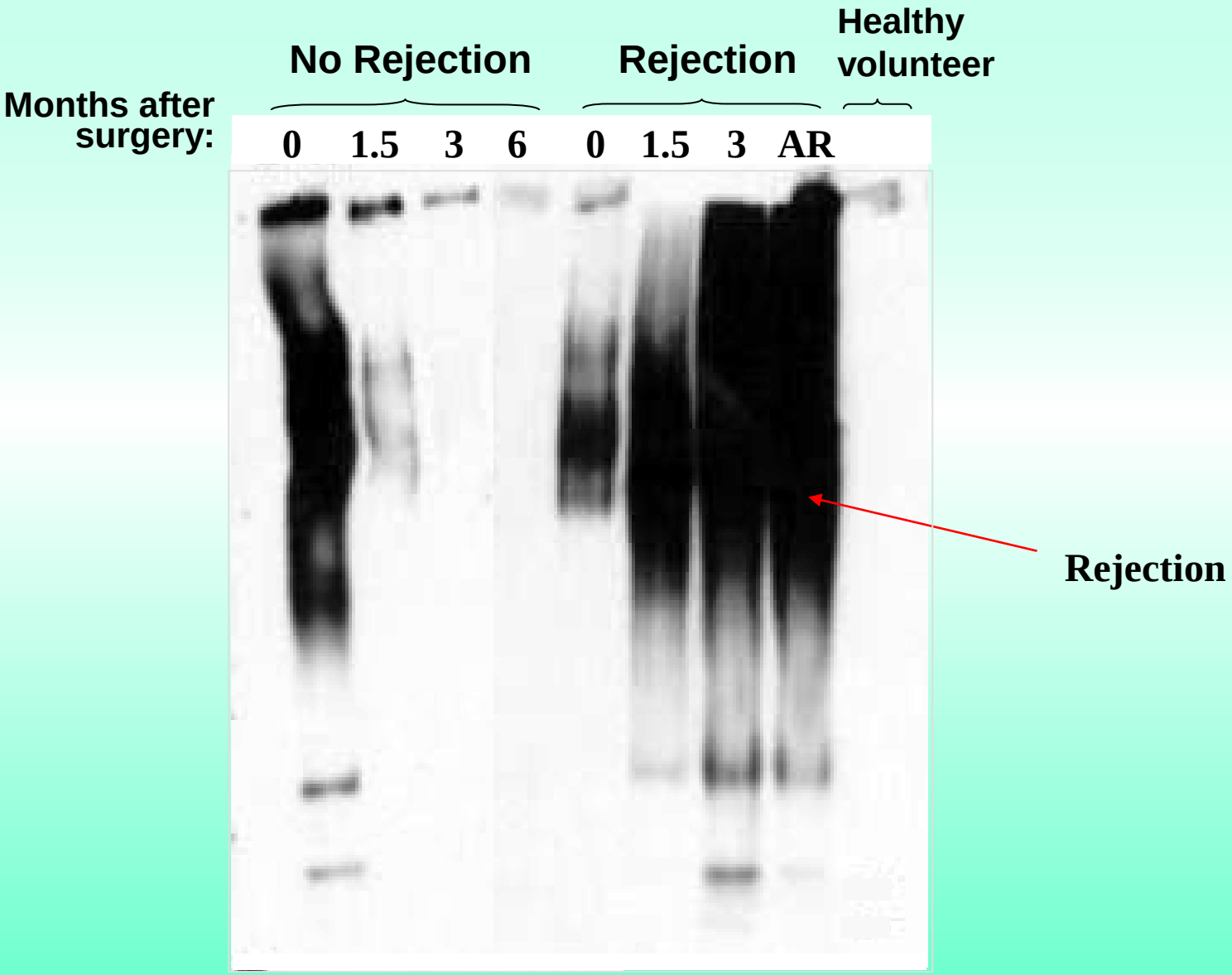
BAL SNO-SP-D content also increases with increasing disease severity in HPS1 patients

E Atochina-Vasserman et al, JCI, submitted, 2010

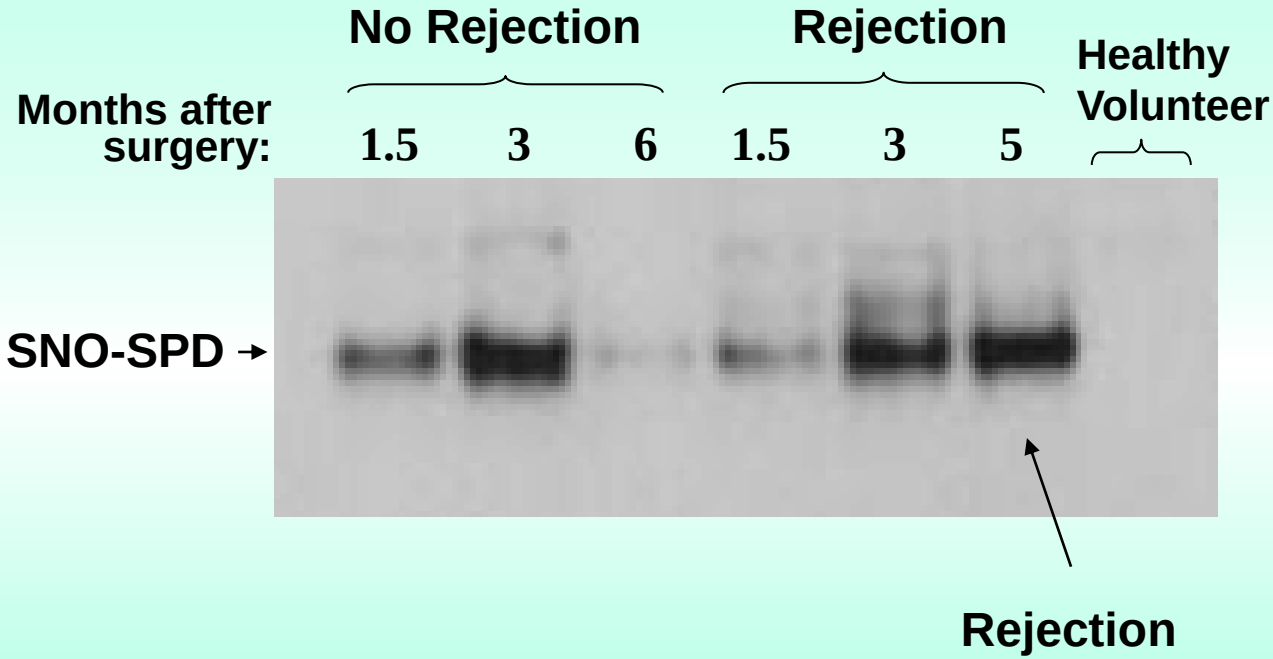
Acute rejection of lung allograft is associated with cross-linking of SP-D



Acute rejection of lung allograft is associated with disruption of SP-D multimers



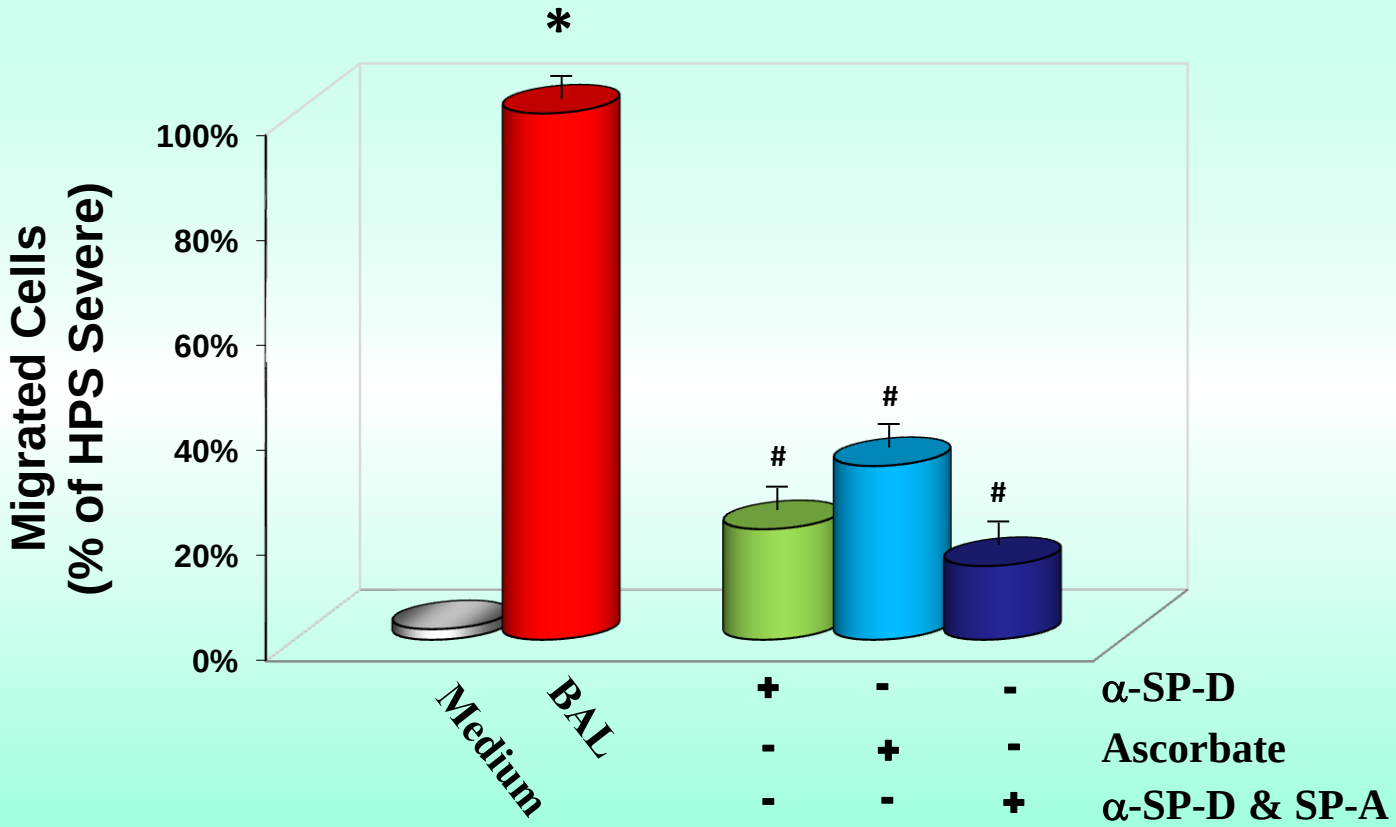
Acute rejection of lung allograft is associated with SNO-SP-D



- **SP-D can be *S*-nitrosylated to form SNO-SP-D by lung inflammation**
- ***S*-nitrosylation of SP-D reduced its multimeric state**

Does SNO-SPD have any functional consequences for lung inflammation?

SP-D modifications in HPS1 patients promote enhanced macrophage migration



E Atochina-Vasserman et al, JCI, submitted, 2010

Summary

- **Lung inflammation induces the production of NO and *S*-nitrosylation of SP-D (SNO-SPD)**
- ***S*-nitrosylation leads to the disruption of the SP-D multimeric structure**
- ***S*-nitrosylated SP-D activates alveolar macrophages through calreticulin and CD91 receptor and could enhance lung injury during different experimental models**
- **Oxidatively cross-linked SP-D is formed in human inflammatory lung disease**
- **SNO-SP-D is formed in human lung disease and is associated with increased inflammatory function.**

Conclusions

Nitrosative and oxidative modifications of SP-D occur in human inflammatory lung disease and these modifications have potentially significant consequences in controlling innate immune function

Acknowledgements

University of Pennsylvania
School of Medicine
Philadelphia, PA

Michael F. Beers

Helen Abramova

Karen Sims

Fraunhofer Institute of Toxicology
and Experimental Medicine
Hannover, Germany

Jens M. Hohlfeld

Carla Winkler

Rutgers University
Piscataway, NJ

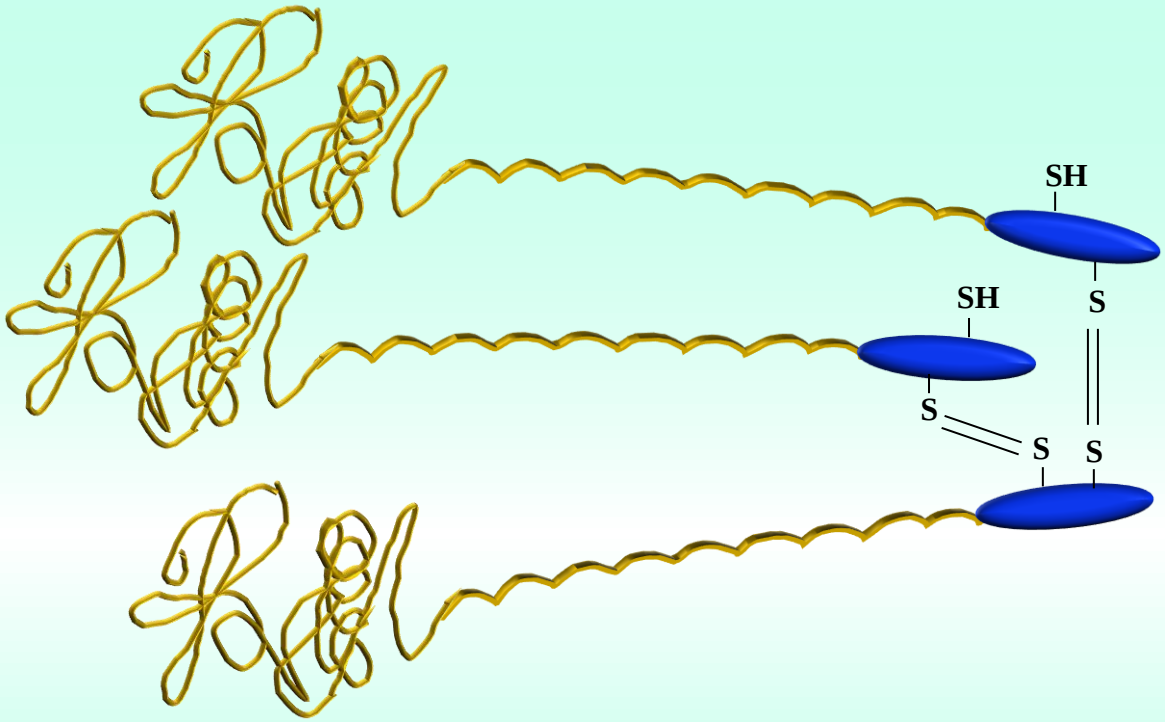
Andrew J. Gow

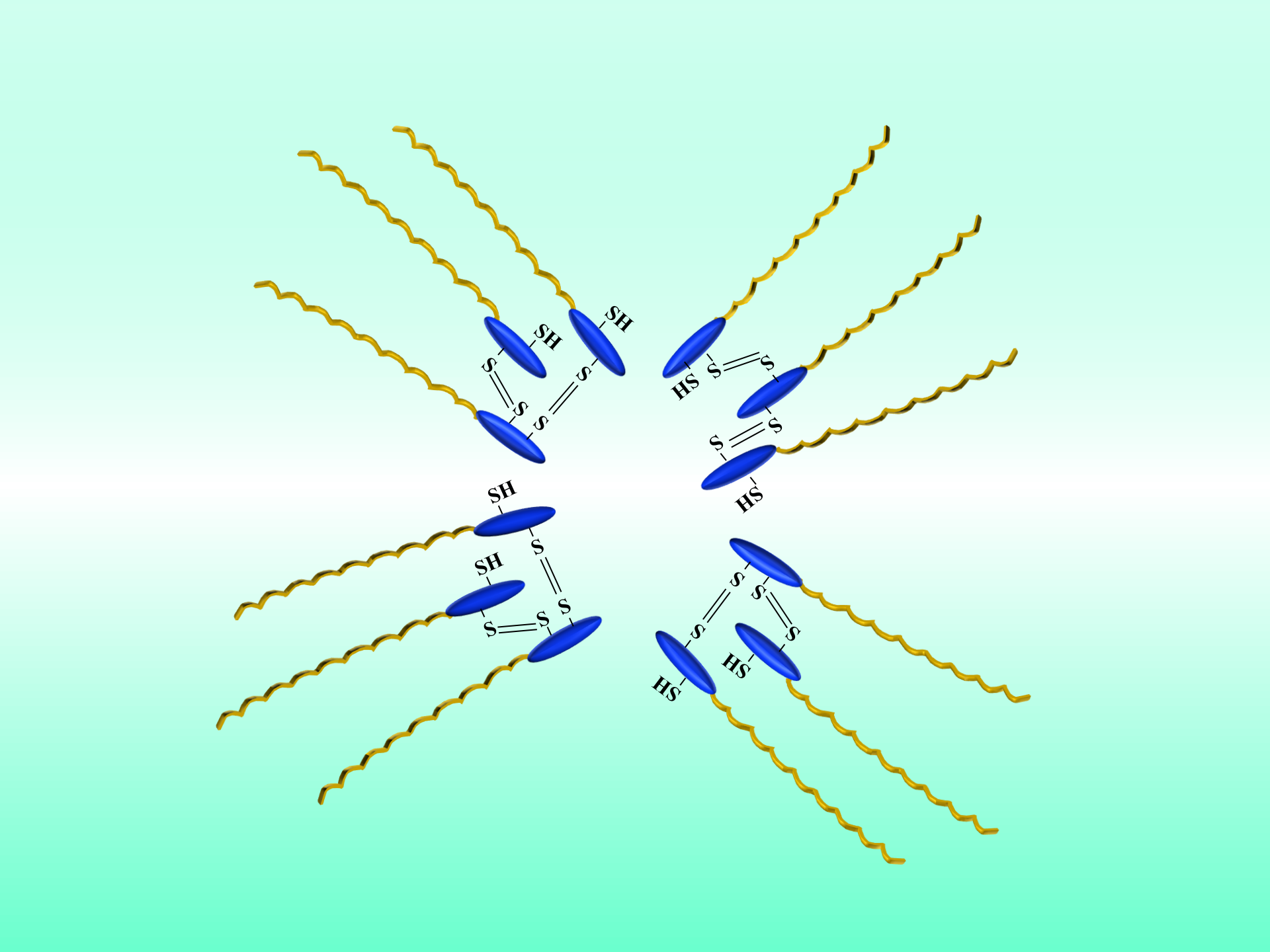
Chang J. Guo

Children's Hospital of
Philadelphia
Philadelphia, PA

Susan H. Guttentag

The HPS network


SH
SH
S
S
S
S


SH
SH
SH
SH
SH
SH
SH
SH
S
S
S
S
S
S
S
S
S
S
S
S
S
S
S
S
S
S
S
S