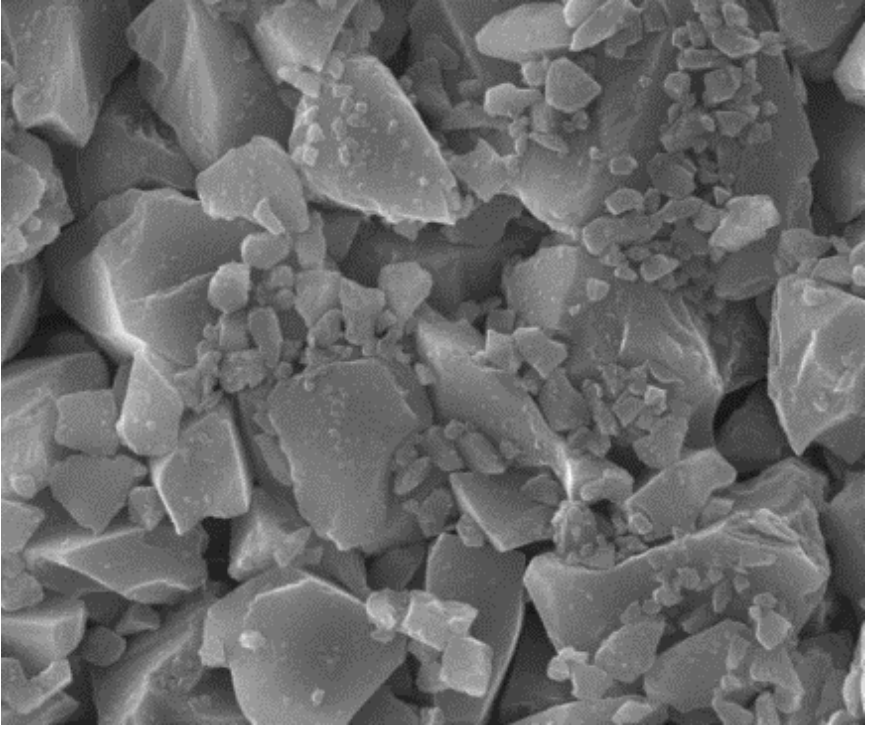
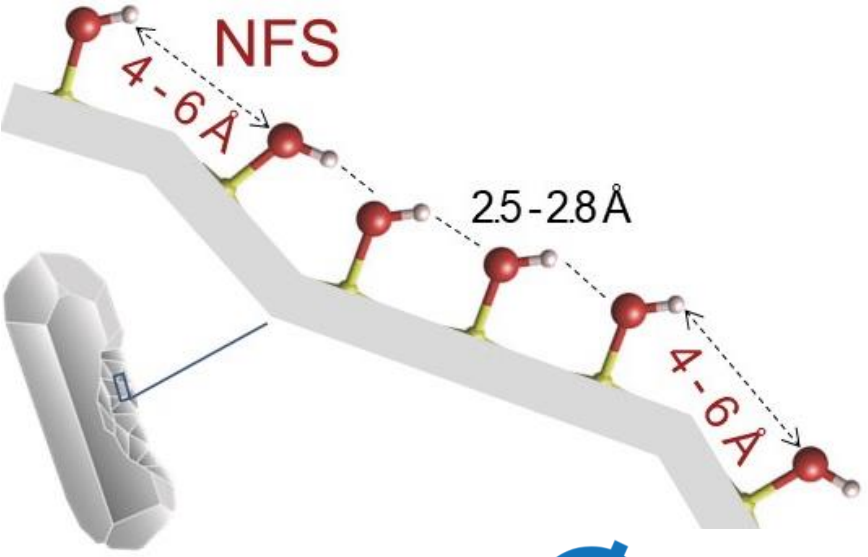


Molecular recognition between membrane epitopes and nearly free surface silanols on silica:

A new paradigm for particle toxicity mechanism



Francesco Turci

«G. Scansetti» Center for Studies on Asbestos and Other Toxic Particulates
Department of Chemistry, University of Turin, Italy

francesco.turci@unito.it

EGU General Assembly 2022 Vienna, Austria & Online | 23–27 May 2022

RCS is the leading cause of occupational respiratory disease worldwide

- The WHO Global Health Report estimated that silicosis causes > 10,000 deaths each year – significant underestimation
- *Global Burden of Diseases 2017 Causes of Death, Lancet 2018*
- Several tens of millions of people in the world are currently exposed to silica dust at work
- 3-5 millions in Europe, ca. 2 millions in USA, 11 millions in India, +20 millions in China


THE LANCET
Respiratory Medicine

Editorial

The world is failing on silicosis

In early 2019, the Australian news network ABC reported the results of an audit of the stonecutting industry in Queensland following the identification of several cases of silicosis in the region in 2018. The results caused substantial concern. After the audit, the government agency Workplace Health and Safety Queensland issued 552 compliance notices across 138 stonecutting premises that were found not to be implementing measures to prevent development of the disease among workers. The Queensland government has also put in place a screening programme; so far, 799 workers have been screened, of whom 98 have silicosis.

Silicosis is caused by the inhalation of respirable crystalline silica dust which, over time, leads to lung inflammation and fibrosis. The condition is progressive and almost always fatal; no specific treatment exists, although a small minority of patients may be offered lung transplantation. Silicosis has also been shown to greatly increase the risk of pulmonary tuberculosis among those affected, with this risk remaining high even when exposure to silica dust has ceased.

The disease is entirely preventable. Interventions such as wet-cutting (which turns airborne silica dust into a slurry), good ventilation practices, and perhaps, most importantly, effective, well fitted supplied-air respirators (but not thin surgical masks), can all protect workers. These measures are known to be effective; for example, there was a substantial decrease in the incidence of laws together with poorly regulated workplaces play a part to some extent. Underlying these issues, however, is a globalised supply chain in which the end consumer is almost entirely removed from the circumstances of production, allowing poor working practices to go unchecked, and maintaining a downward pressure on prices and therefore wages.

Of the few reports on silicosis, including the recent Australian audit, most circle back to education as a tool in the fight against the disease. It is likely that many people worldwide who are exposed to silica dust in their workplace are unaware of the very real danger it poses. It is also possible that many employers are not aware of the risk. Education is therefore key in any global or national effort to combat the condition.

There is also an absence of strong leadership on the issue. In 1995, WHO and the International Labour Organization began a public awareness and prevention campaign to eliminate silicosis from the world by 2030. The campaign garnered some support, and led to the formation of several national programmes, including in China, South Africa, and Turkey, aimed at eradicating the condition. Unfortunately, the campaign does not appear to have maintained momentum. There have been no identifiable national level follow-up studies or centralised follow-ups to the original report. A 2015 study of South African gold miners found no decrease in the prevalence of silicosis in that population between 1984 and 2009.

Cullinan et al., *Lancet Respir Med*, **2017**

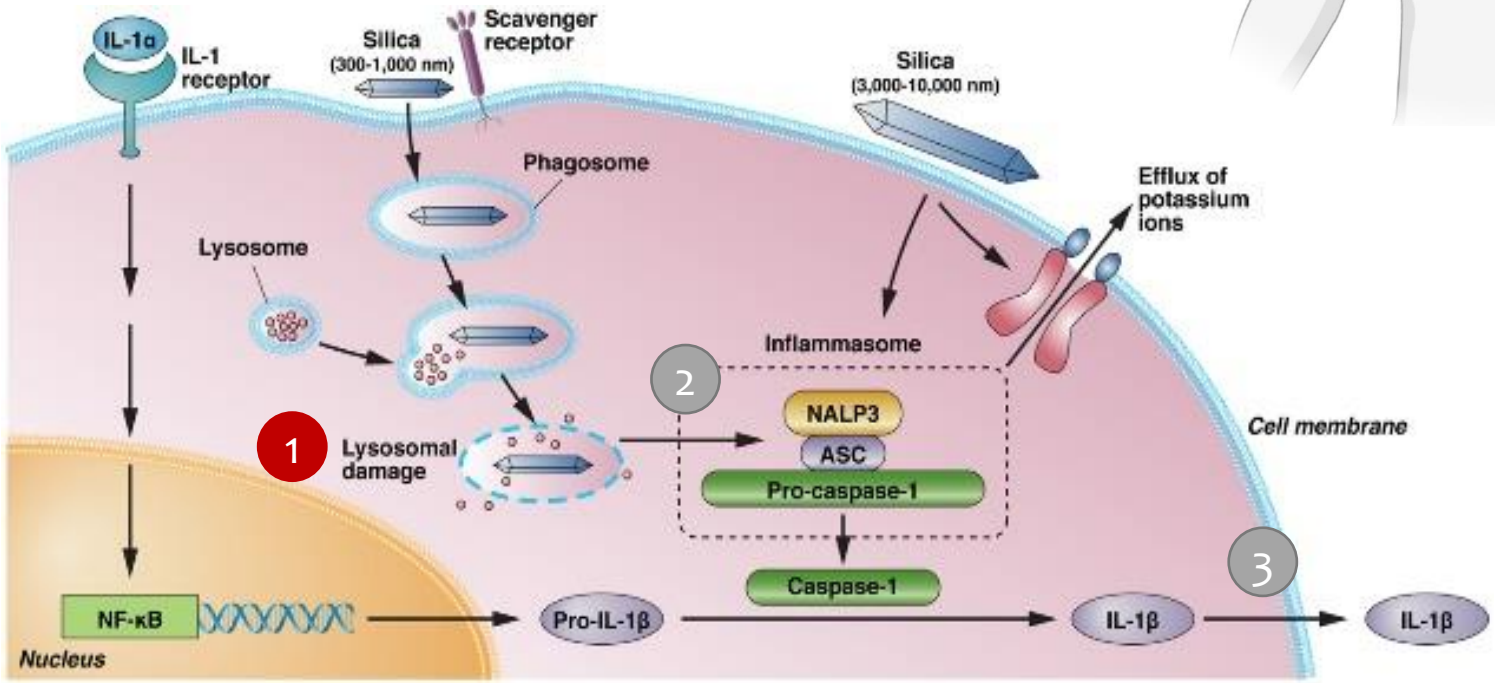
IARC, *Monograph Vol. 100C*, **2012**

IARC, *Monograph Vol. 68*, **1997**

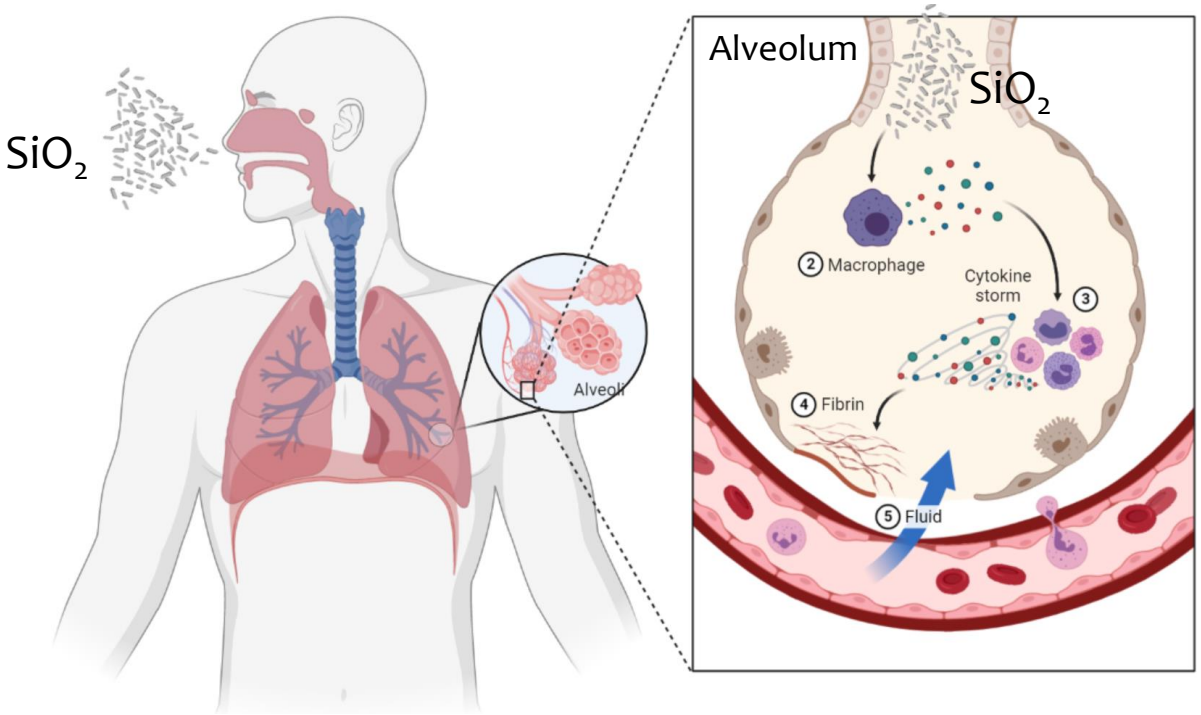
International Agency for Research on Cancer
World Health Organization

Quartz damages membranes: the molecular initiating event (MIE)

Dostert et al. Science 2008
Hornung et al. Nat. Immunol. 2008
Cassel et al. PNAS 2008



Adapted from Pollard Front. Immunol. 2016

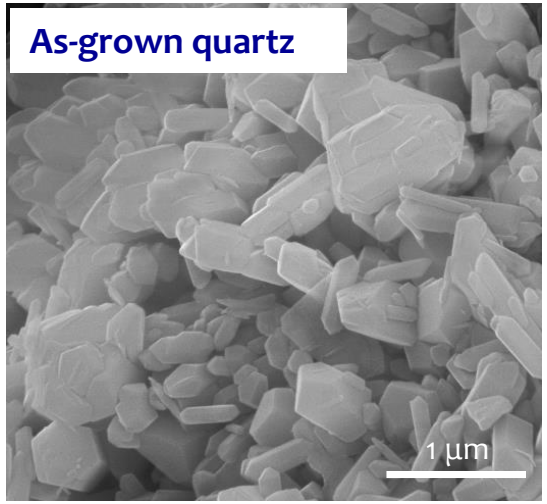


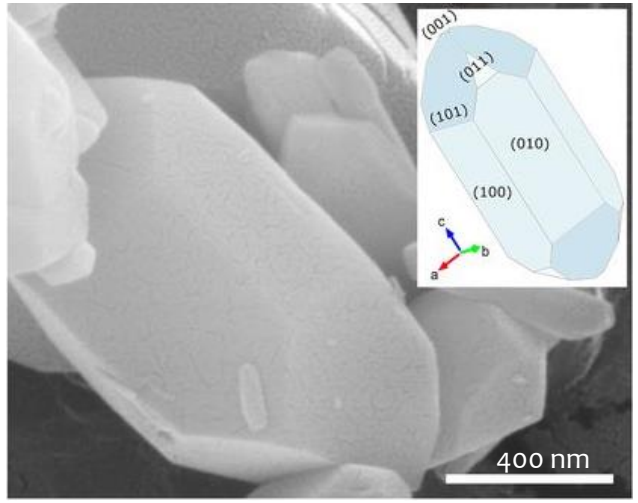
1. **Phagolysosome membrane damage**
2. Inflammasome activation
3. IL-1β release from macrophages

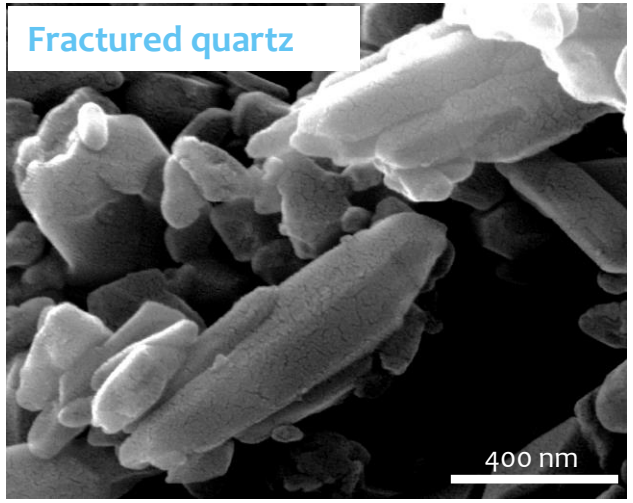
Inflammation

Fractured quartz damages cell membranes in RBC model

Silica model: **as-grown α-quartz crystals in respirable size** obtained by hydrothermal synthesis for toxicological tests

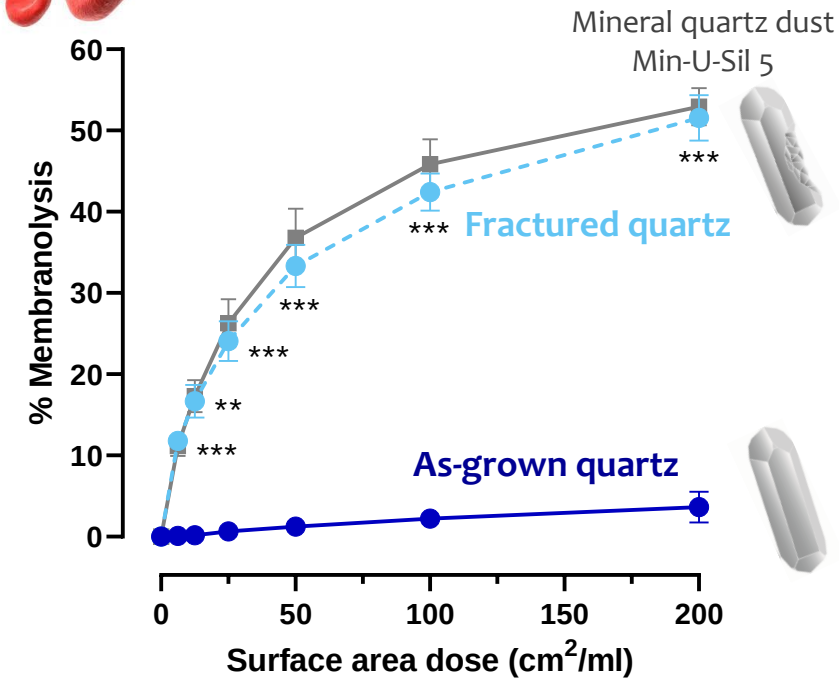






Membranolytic activity

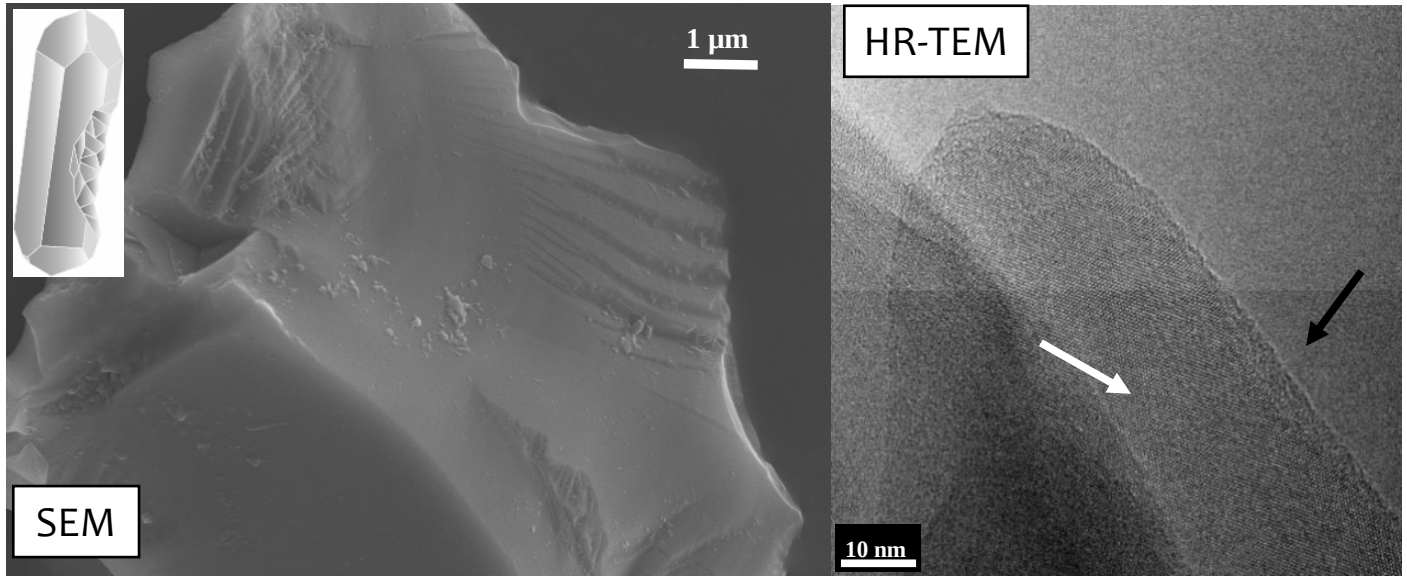
Red blood cells as *model of cell membranes*

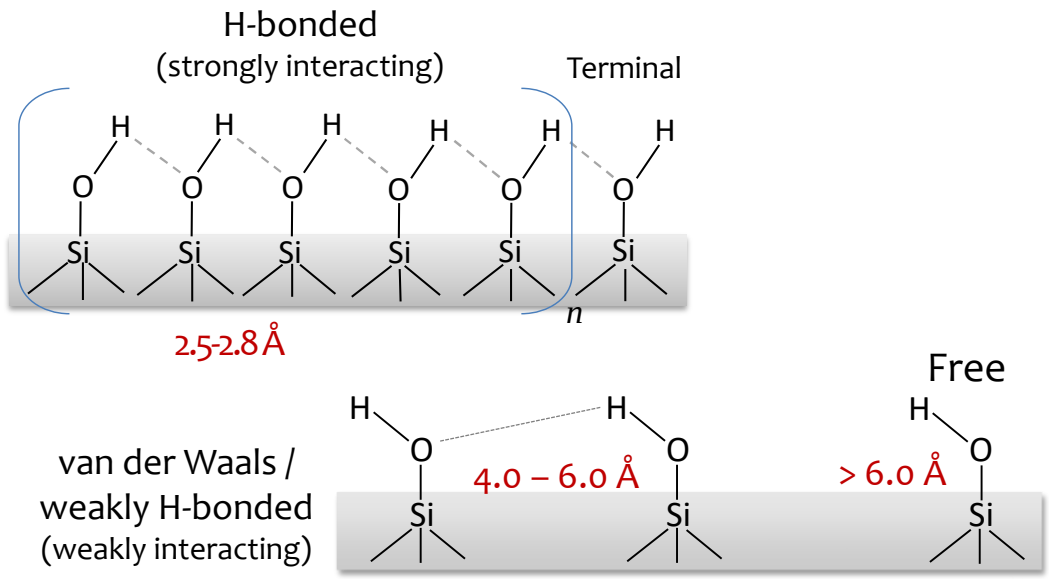


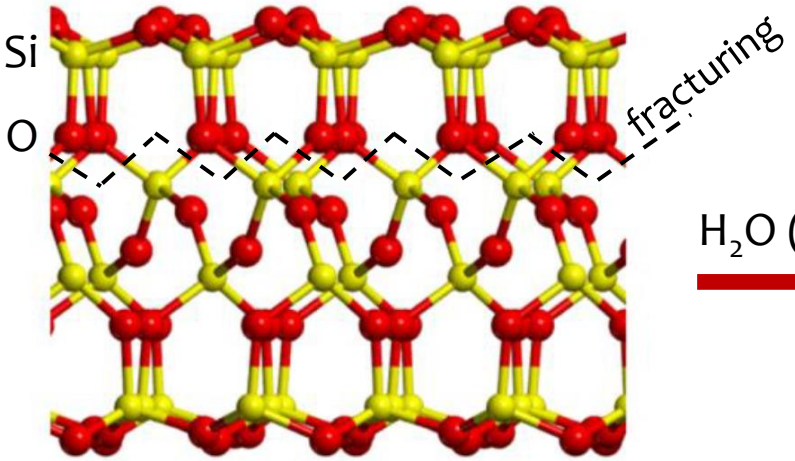
- New synthetic approach delivers **as-grown quartz** with **no membranolytic activity**
- As-grown quartz crystals showed **membranolytic effects after fracturing**

Pastero et al. *Cryst Growth Des* 2016
Turci et al. *Part Fibre Toxicol* 2016

Fracturing silica: surface reconstruction and silanol formation



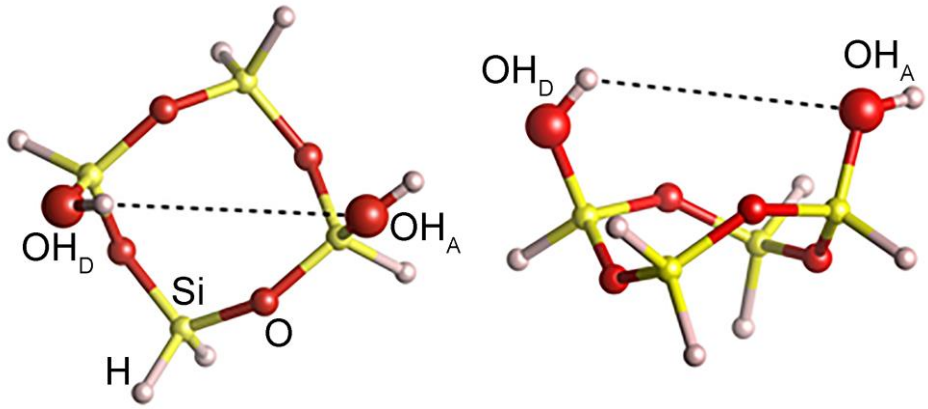




Crystalline silica bulk

H_2O (vap)

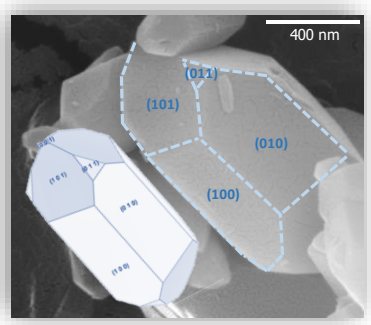
Silanols are formed

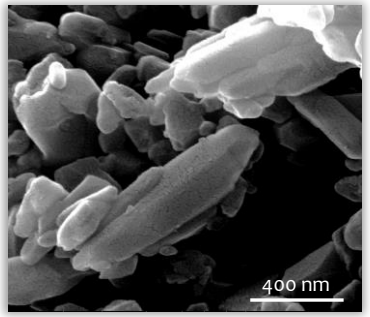


- Different silanol families exhibit different reactivity
- A peculiar family with inter-silanol distance between 4-6 Å was evidenced on fractured Qz
- **Nearly Free Silanol (NFS)**

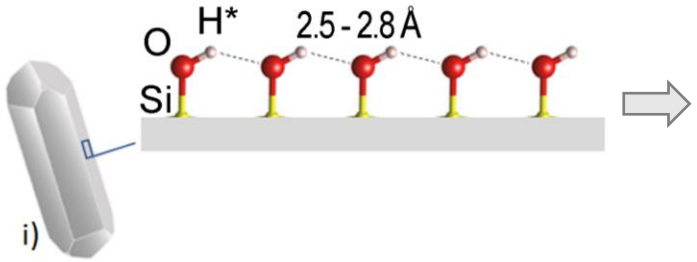
Rimola et al. Chem Rev 2013
Pavan et al. Part Fibre Toxicol 2019

“Nearly-free silanols” trigger the toxicity of silica particles



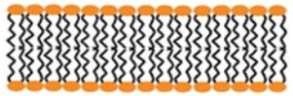


As-grown

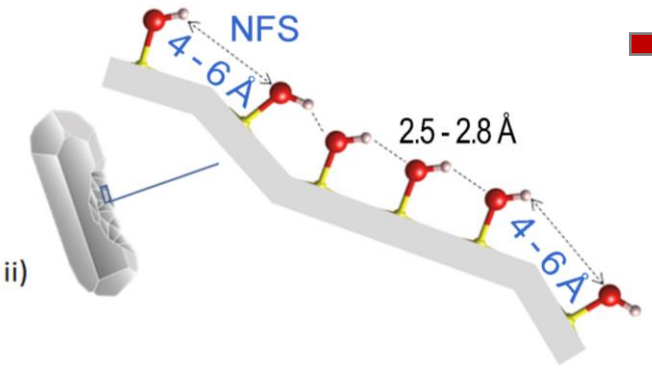


In vitro and *in vivo*

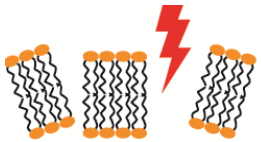
Non membranolytic
Non inflammogenic



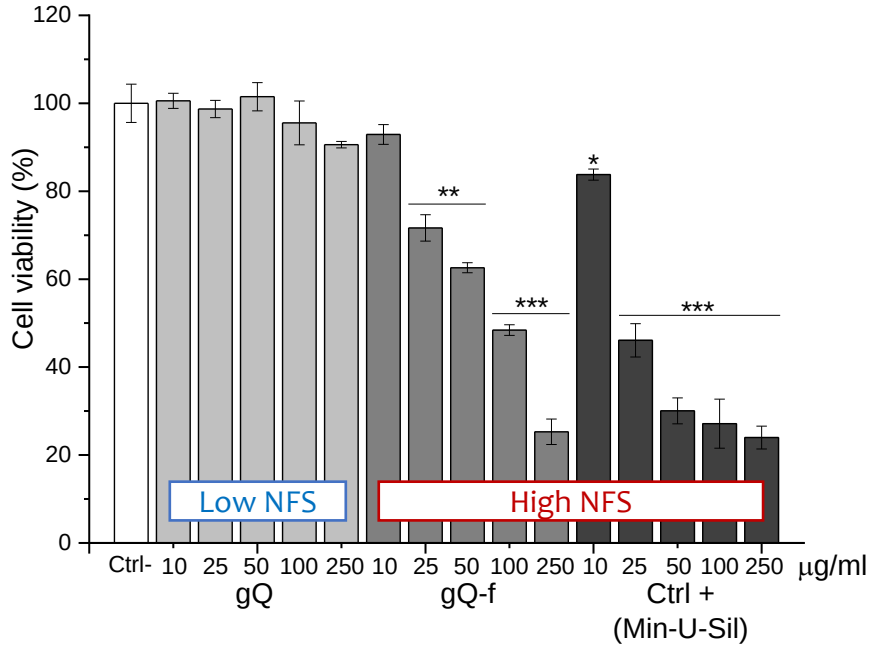
Fractured



Membranolytic
Inflammogenic



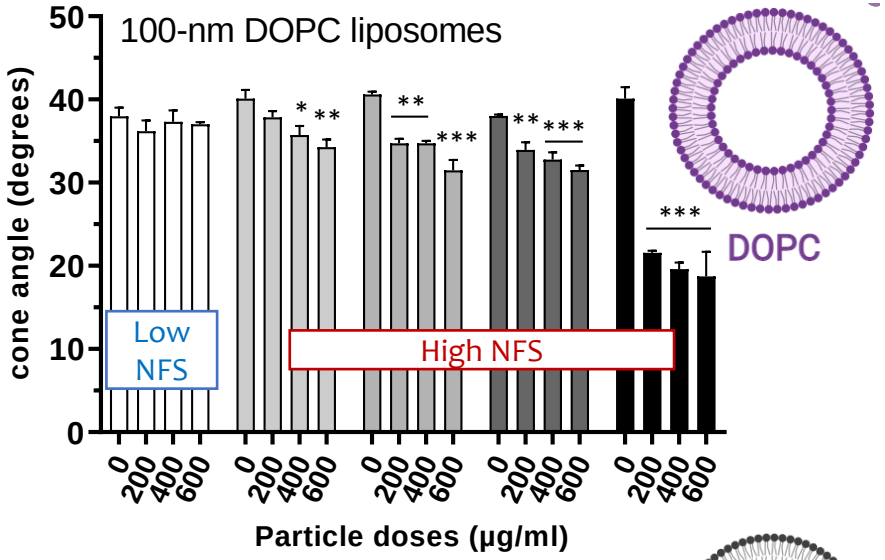
- Fracturing induces the formation of a peculiar silanol group, the **NFS**
- The quantitative variation of the NFS is related to the activation of **early toxic responses**

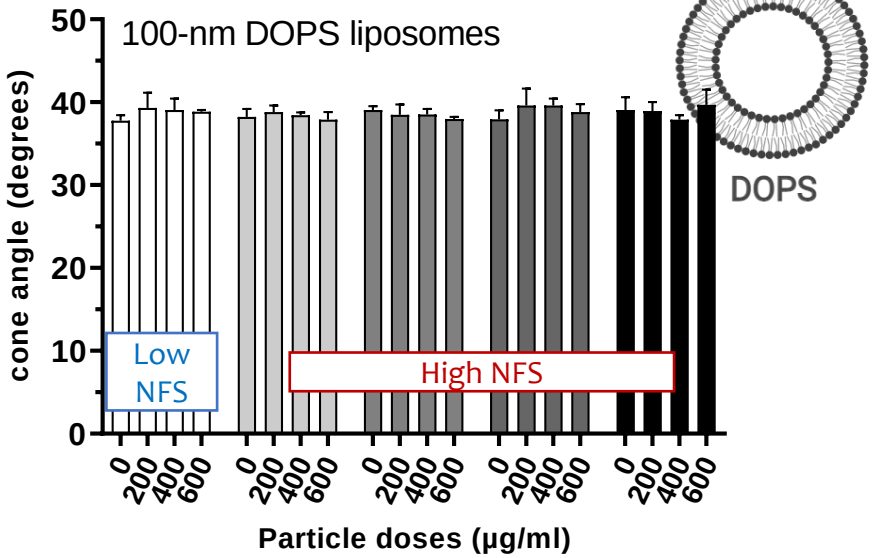


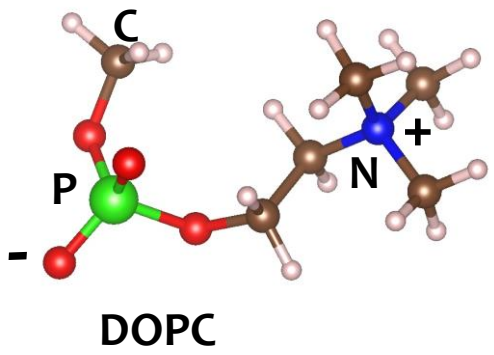
- **NFS geometry** maximizes the interaction energies with phosphate groups of cell membranes
- Causal relationship between **NFS** and **membranolytic** effect was proved for **quartz, cristobalite, and amorphous silica**

Pavan C., Santalucia R., Linardi R., Fabbiani M., Yakoub Y., Uwambayinema F., Ugliengo P., Tomatis M., Martra G., Turci F., Lison D. & Fubini B., ***Proc. Natl. Acad. Sci. USA***, 117 (45): 27836, 2020

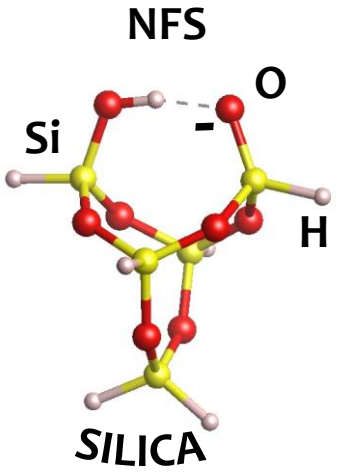
Molecular Recognition Energetics Between Membrane Epitopes and NFS Explain Liposome Selectivity

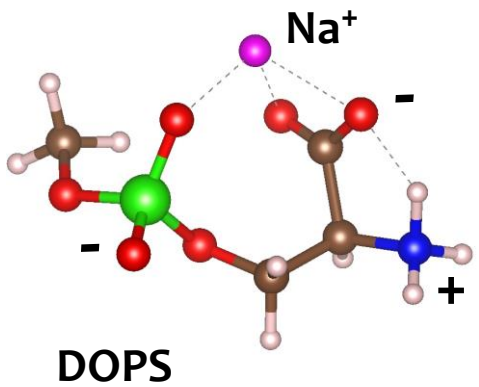




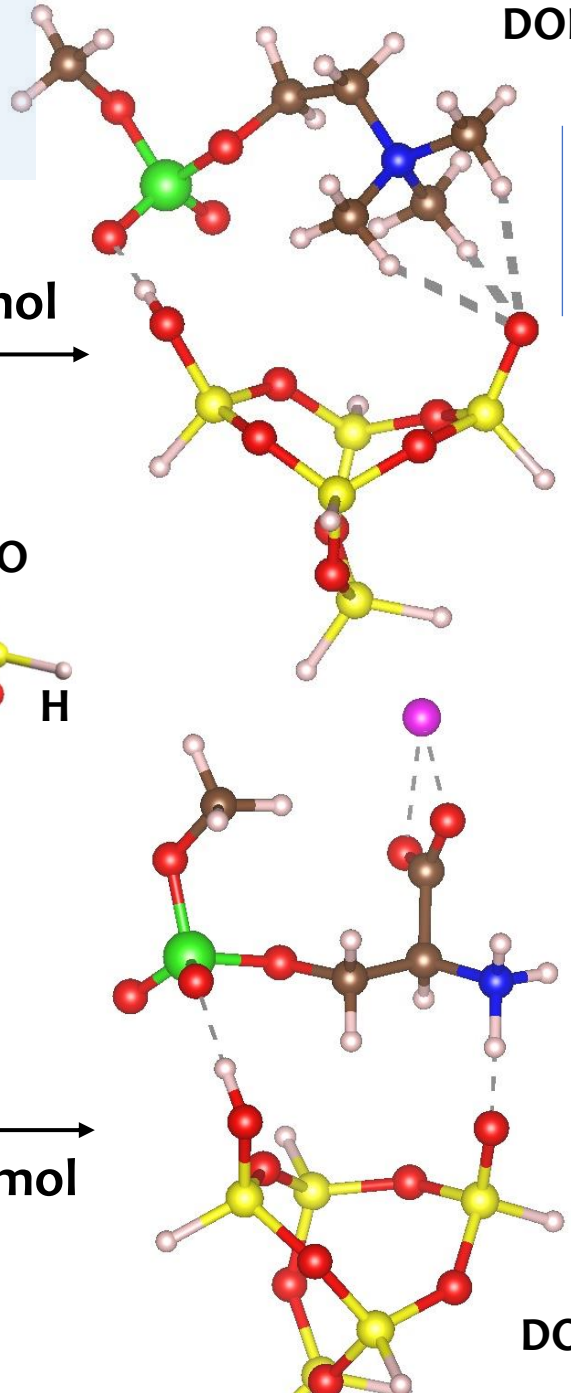


ΔE -32 kJ/mol





ΔE -19 kJ/mol



Cristina Pavan, Matthew J. Sydor, Chiara Bellomo, Riccardo Leinardi, Stefania Cananà, Rebekah L. Kendall, Erica Rebba, Marta Corno, Piero Ugliengo, Lorenzo Mino, Andrij Holian, and Francesco Turci, *Colloids and Surfaces B: Biointerfaces*, Submitted

CONCLUSIONS & FUTURE CHALLENGES

- **Fracturing** quartz crystals upsets the long-range order of surface silanols and introduces **NFS.**
- **NFS**-rich surfaces cause RBC **membrane lysis,** perturbed **liposomes** and **adsorbe PLS**.
- **Specific amino groups** are proposed as **recognition epitopes** for the interaction with NFS
- New perspectives for tailoring **less toxic and improved silica** particles are envisaged

Acknowledgements

UNIVERSITÀ DEGLI STUDI DI TORINO

chimica

Centro Interdipartimentale Amianti G. Scansetti – Università di Torino

Università degli Studi di Torino NIS CENTRE FOR NANOSTRUCTURED INTERFACES AND SURFACES

"G. Scansetti" Centre for Studies on Asbestos and Other Toxic Particulates

Cristina Pavan, Jasmine R. Petriglieri, Ingrid Corazzari, Chiara Bellomo, Valeria Lagostina, Stefania Cananà, Giuseppe Chilla, Maura Tomatis, Cecilia Gomiero, Bice Fubini

Collaborators:

Rosangela Santalucia
Marco Fabbiani
Erica Rebba
Lorenzo Mino
Guillermo Escolano-Casado
Marta Corno
Piero Ugliengo
Linda Pastero
Maria Cristina Paganini

UCLouvain IREC

Yousof Yakoub,
Francine Uwambayinema
Riccardo Leinardi
Sybille van den Brule
François Huaux
Dominique Lison

UNIVERSITY OF MONTANA

Matthew Sydor
Andrij Holian

EUROSIL