New MR Probes to Monitor Active Fibrogenesis

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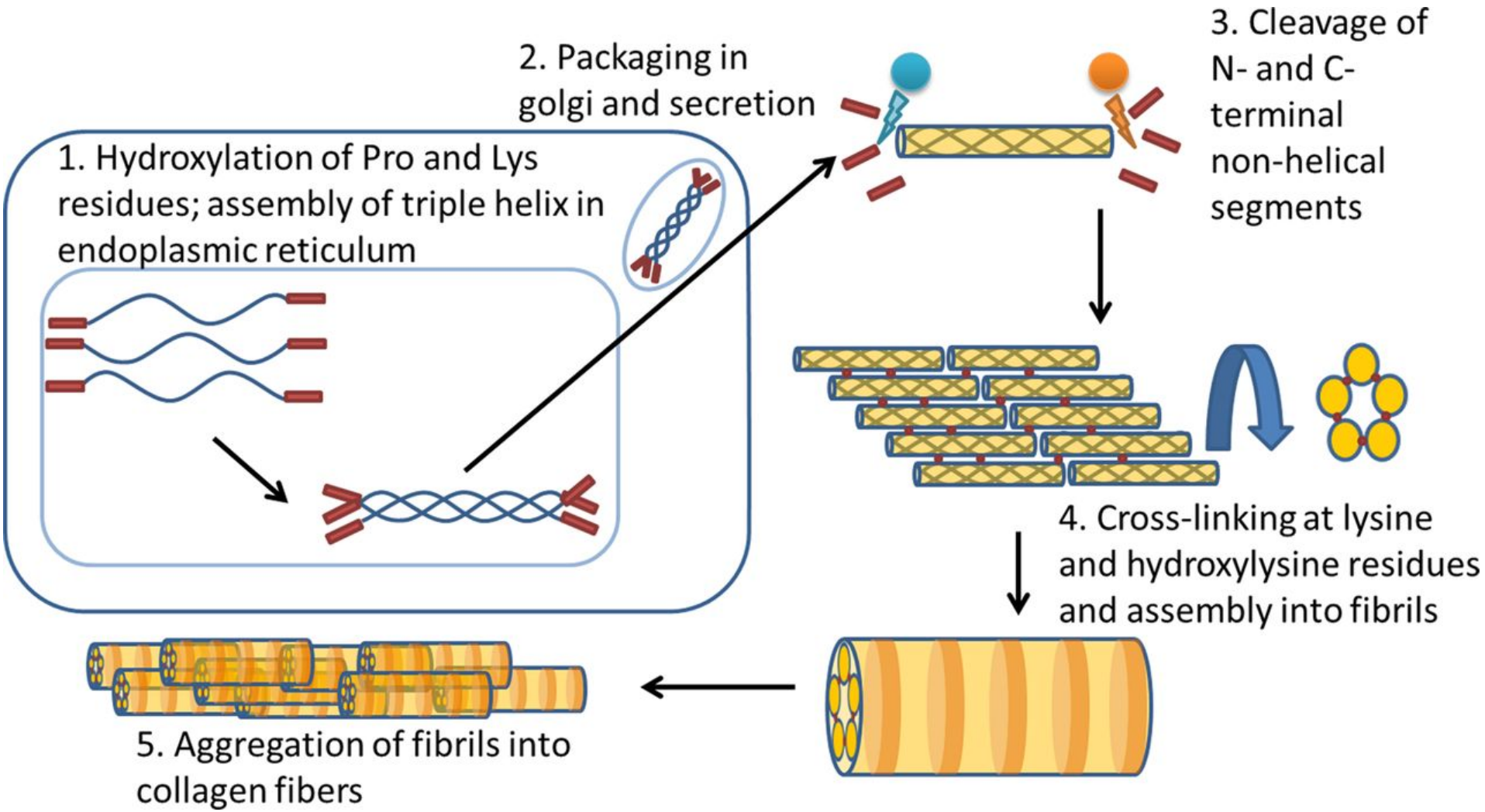
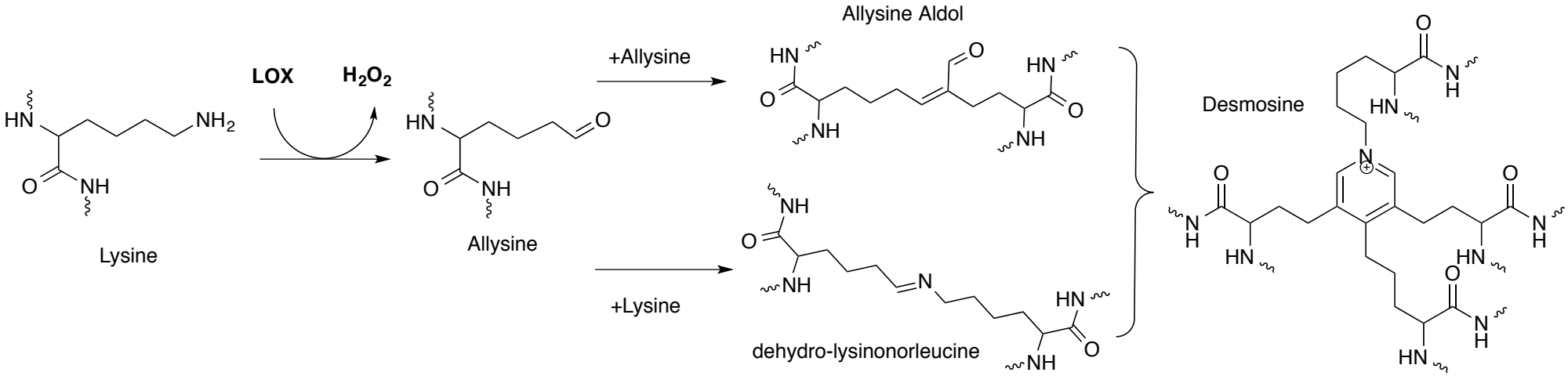
Lysyl oxidases mediate collagen crosslinking – an active process during fibrogenesis



Targeting Fibrogenesis

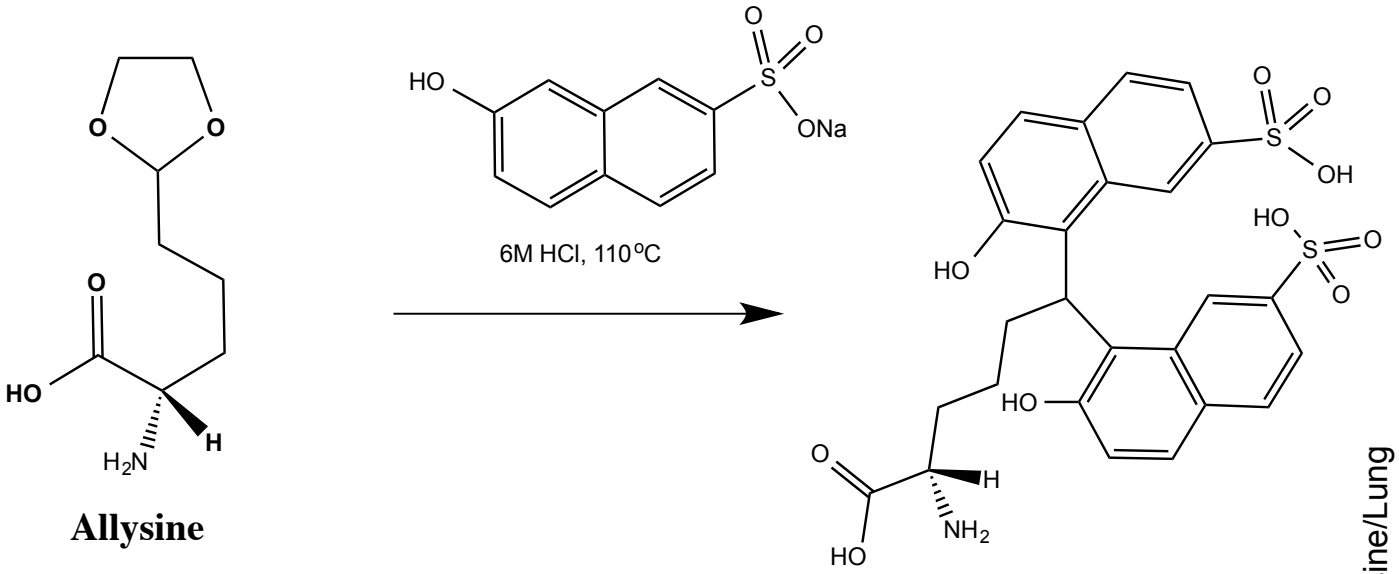
- Active process of fibrotic tissue formation
- Oxidized lysines a product of LOX action on collagen



Hypothesis:

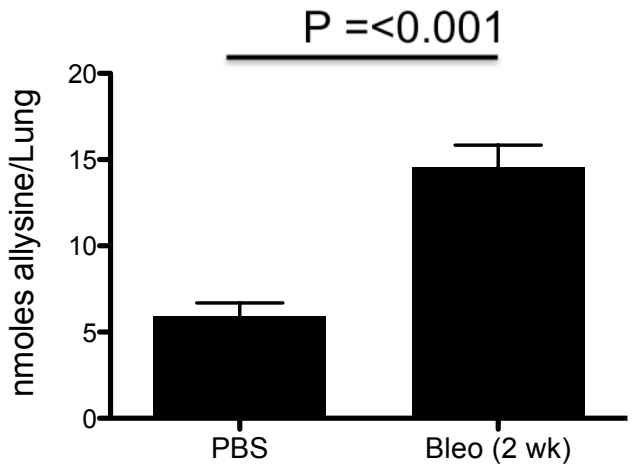
- **Development of a contrast agent that targets oxidized lysine will allow quantification of fibrogenesis by MRI**

Quantification of allysine in tissue

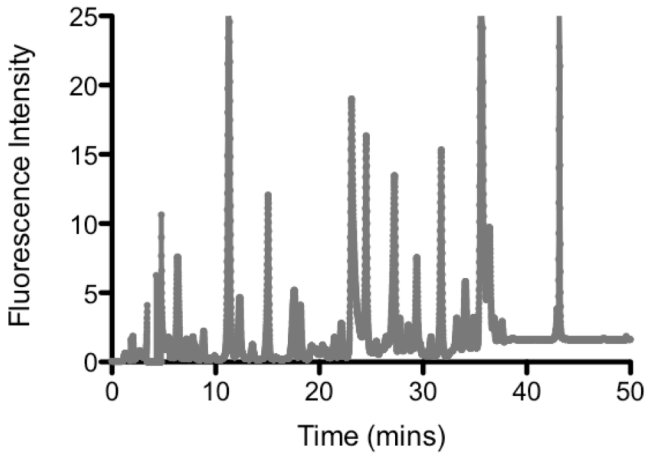


Isolated by prep HPLC and characterized by NMR and LC-MS

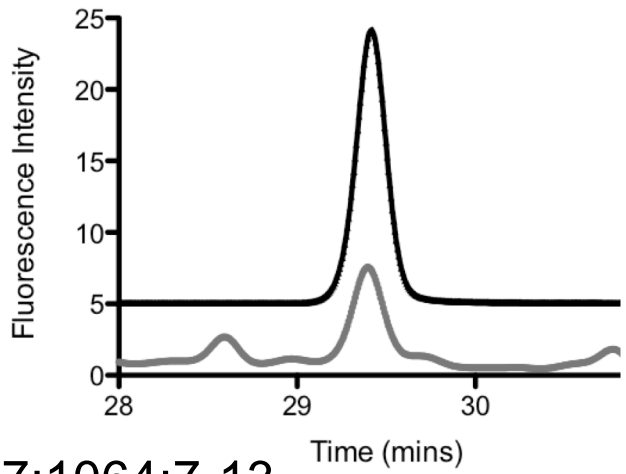
Allysine in lung

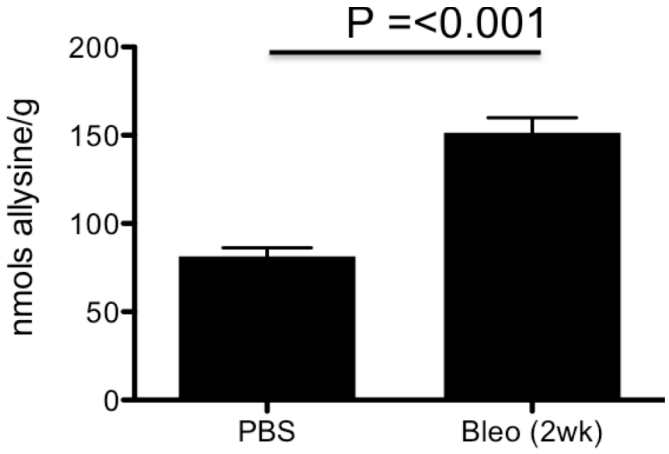


Lung tissue digest



Tissue digest w. standard



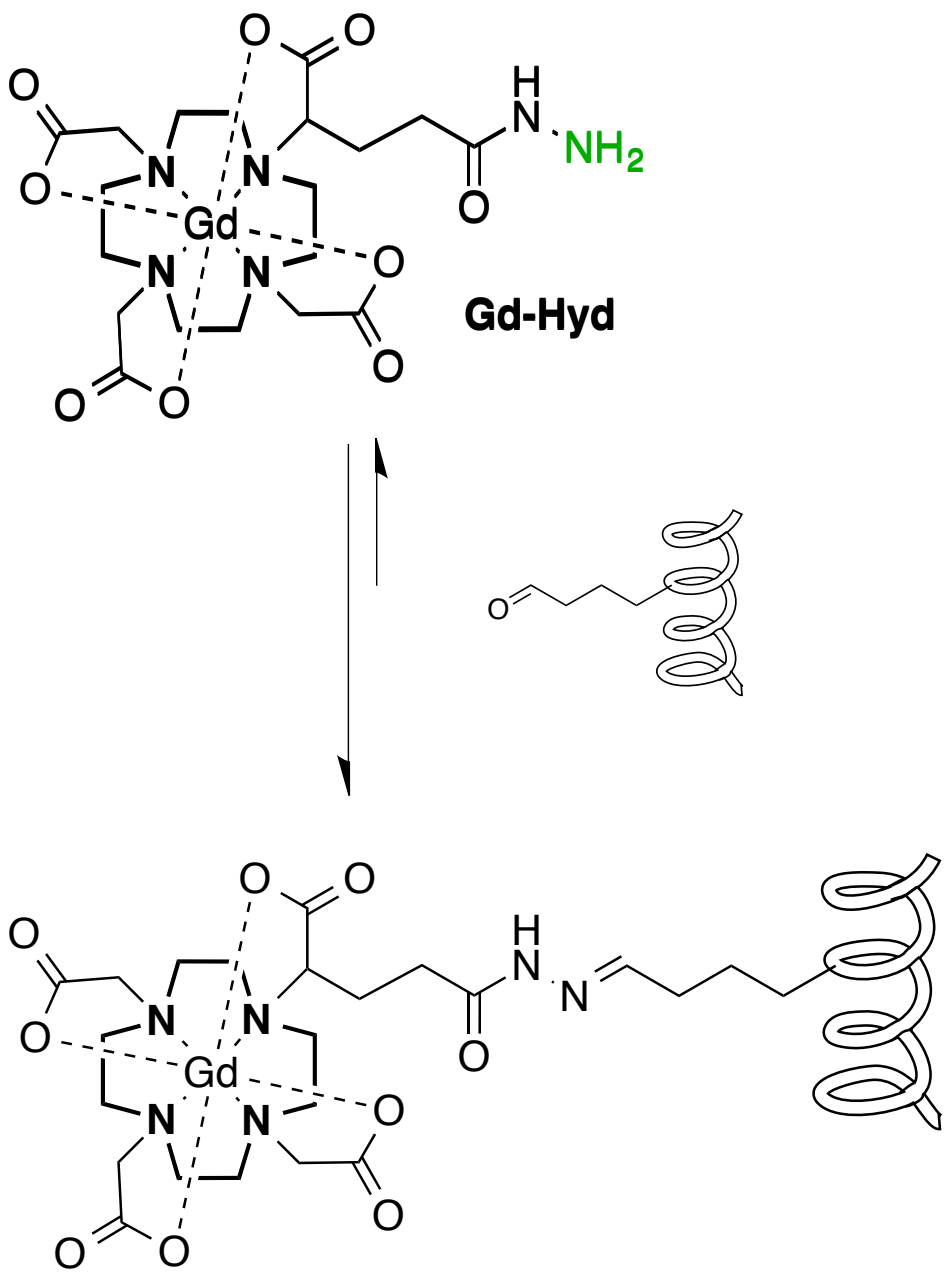


Waghorn, J Chromatogr B. 2017;1064:7-13.

Gd-Hyd – a MR probe that detects oxidized collagen

Desired properties Contrast Agent Design:

- Stable chelate
- Fast blood clearance
- Low non-specific lung uptake
- Hydrophilic and anionic, reduces non-specific binding
- Clearance through renal elimination
- Target selectivity



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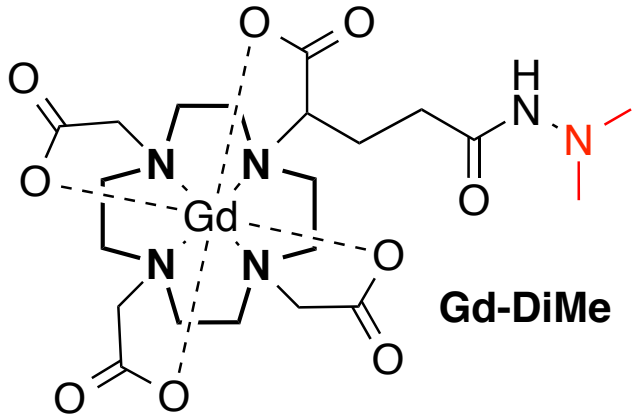
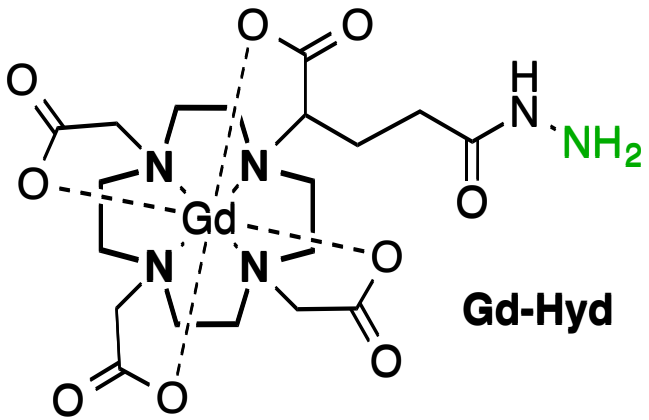
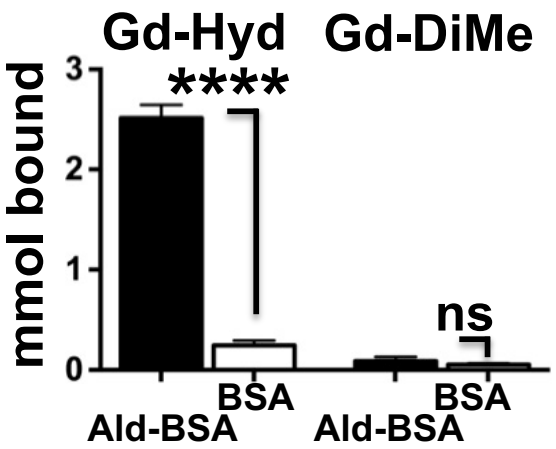
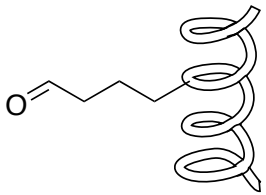
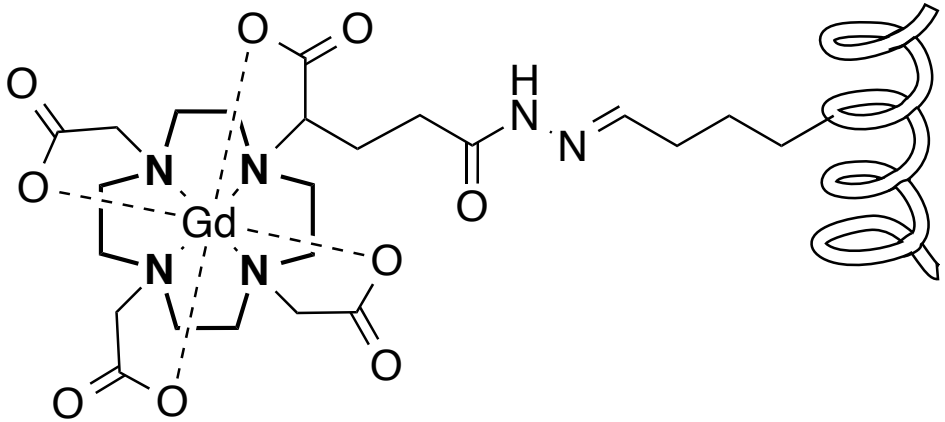
Gd-Hyd – a MR probe that detects oxidized collagen











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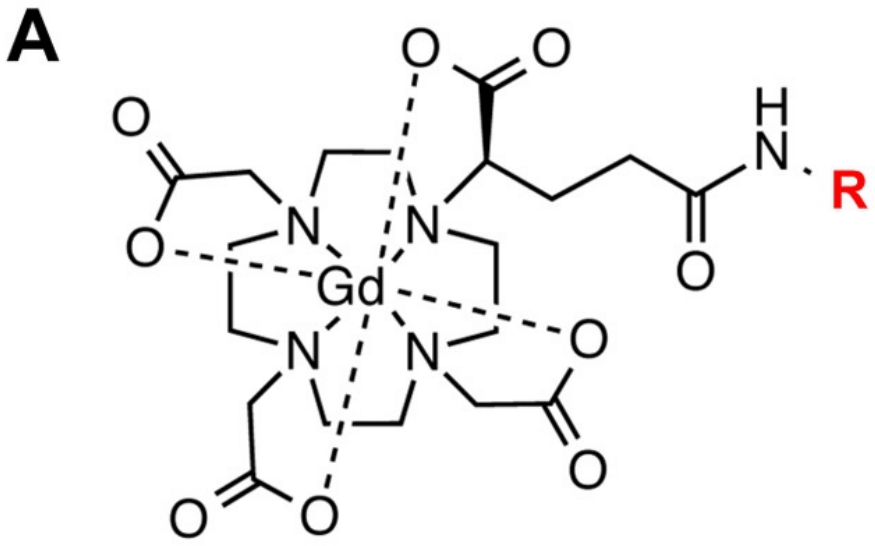
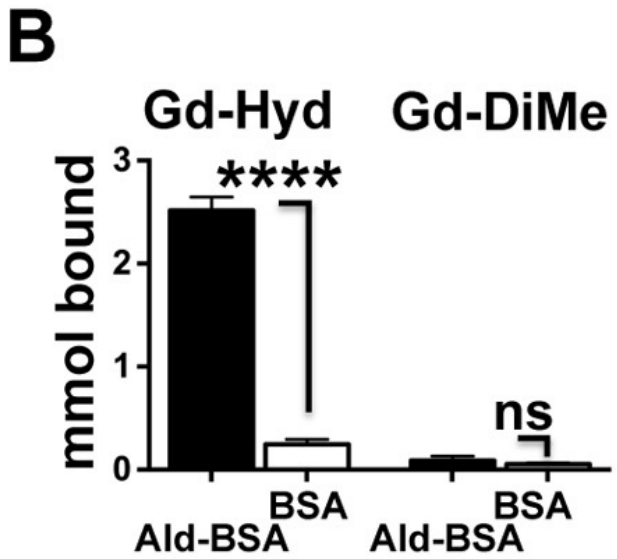
Gd-Hyd binds oxidized lysine

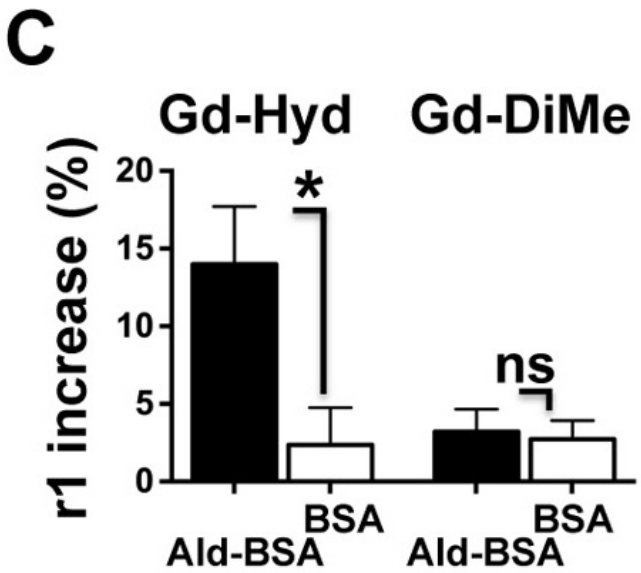


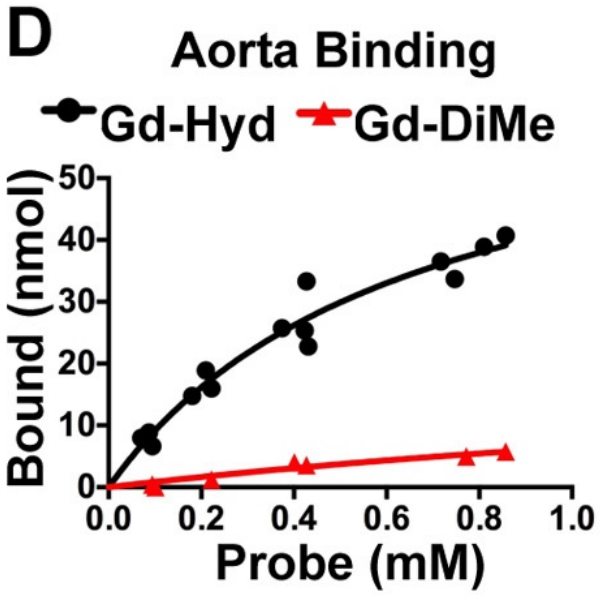
Molecular Probes

Gd-Hyd
R=NH_2

Gd-DiMe
R=$N(CH_3)_2$



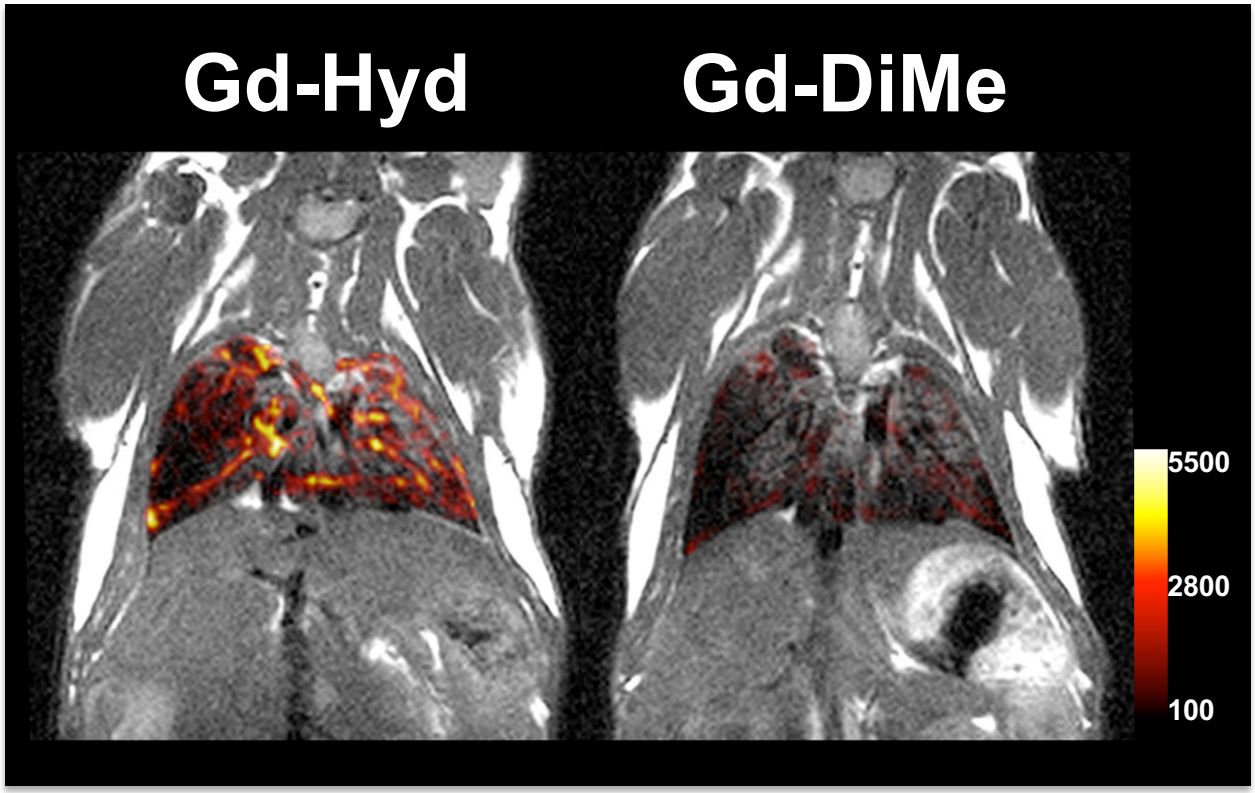


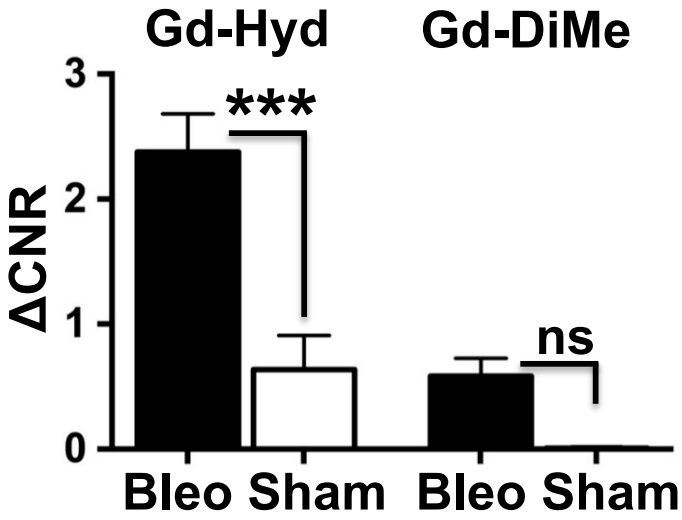


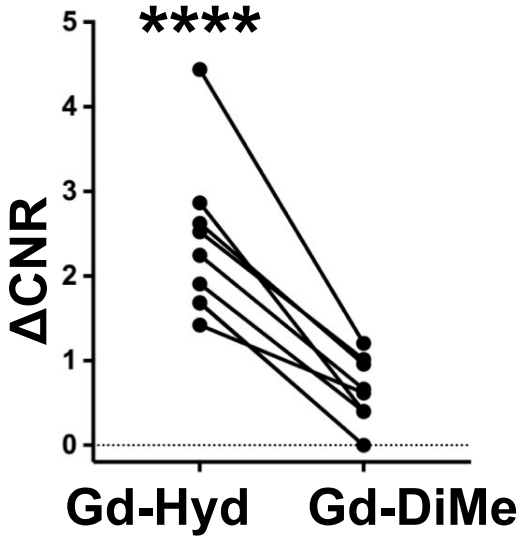
Imaging pulmonary fibrosis/fibrogenesis

- Standard bleomycin model of pulmonary fibrosis in mice
- Transtracheal bleomycin instillation (or vehicle) followed by imaging and ex vivo analysis at day 7 and day 14
- Evaluate effect of pan-LOX inhibitor BAPN on fibrosis
- Compare active fibrogenesis (2 week post bleomycin) to stable scar (4 week post bleomycin)
- UTE-MRI before and 10 min post Gd-Hyd probe
- Ex vivo: histology, hydroxyproline, LOX activity, allysine (LOX oxidized lysine) content

Gd-Hyd displays target selectivity in vivo







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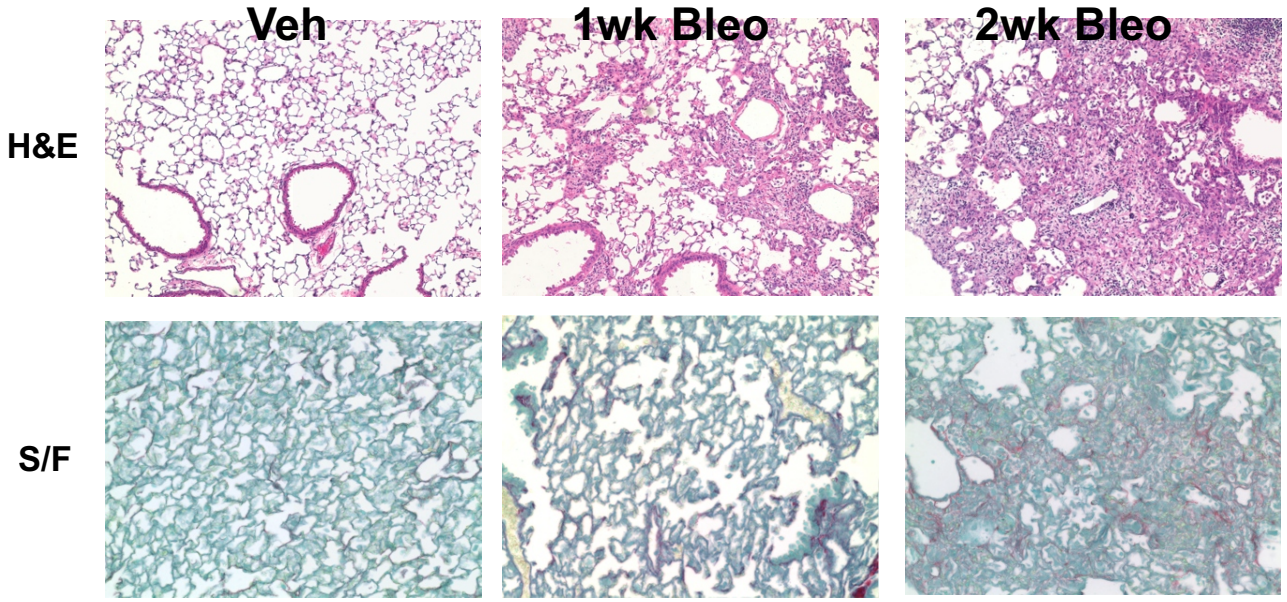
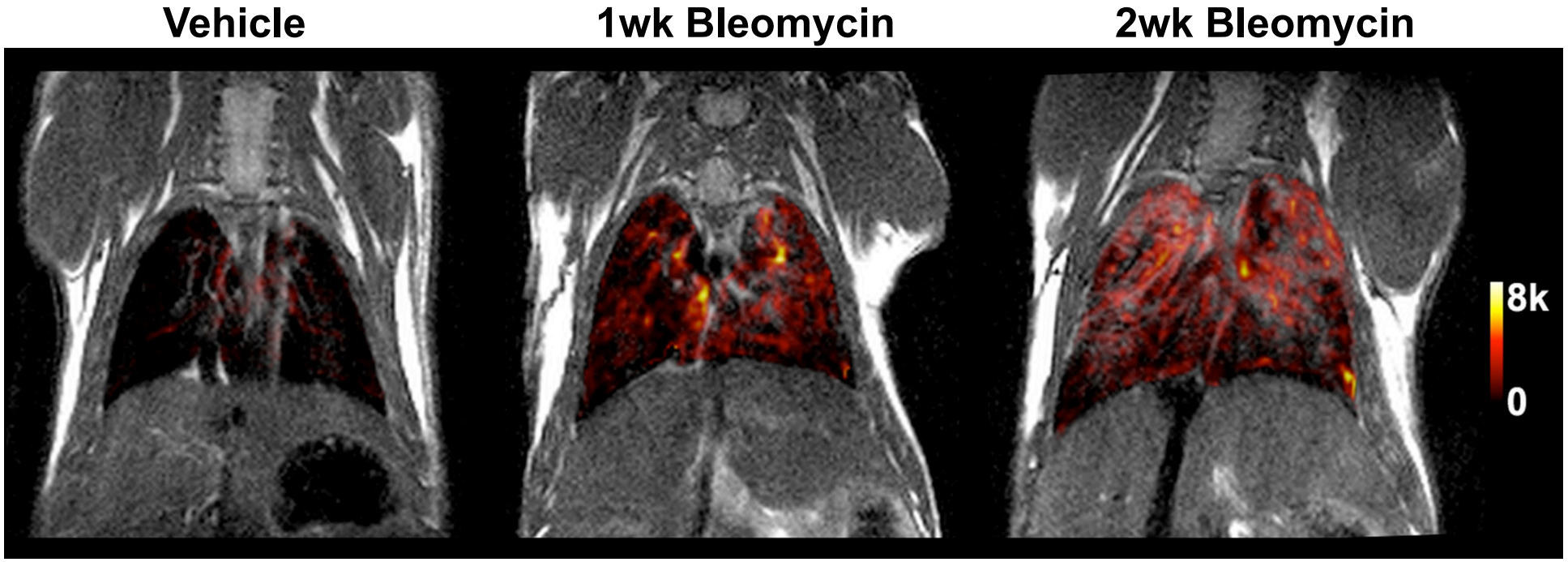
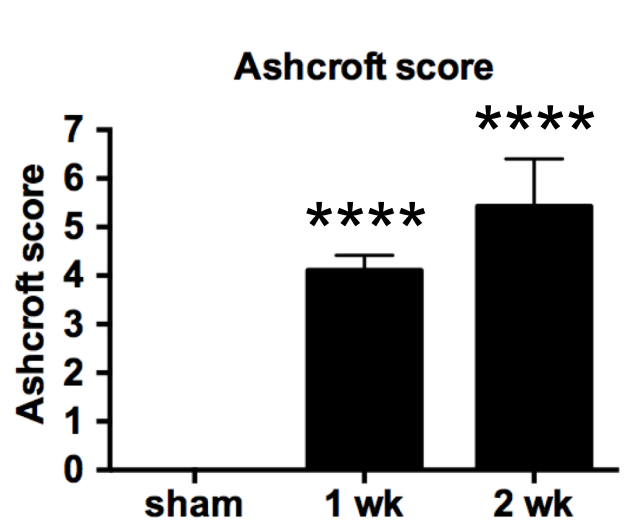
Statistics: *** (P≤0.001), **** (P≤0.0001)

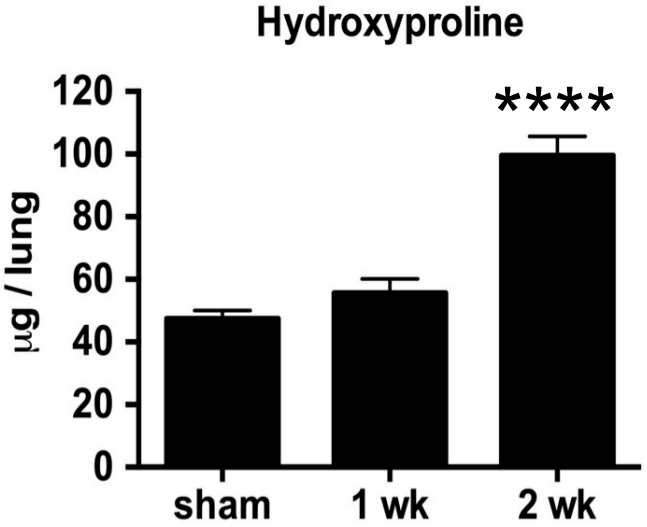
Gd-Hyd allows imaging of disease progression

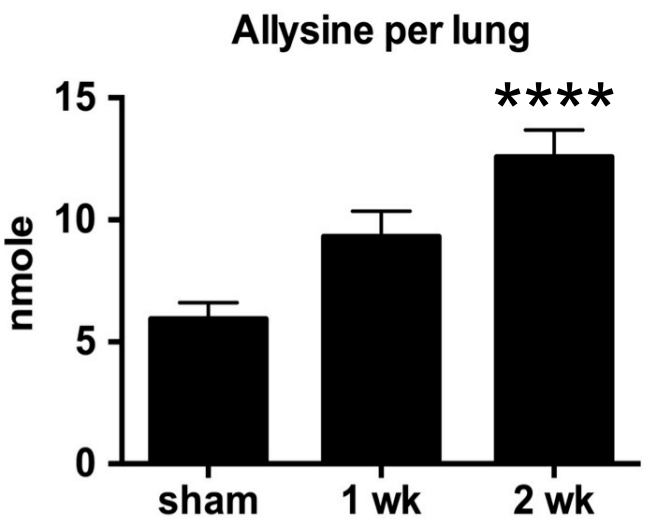


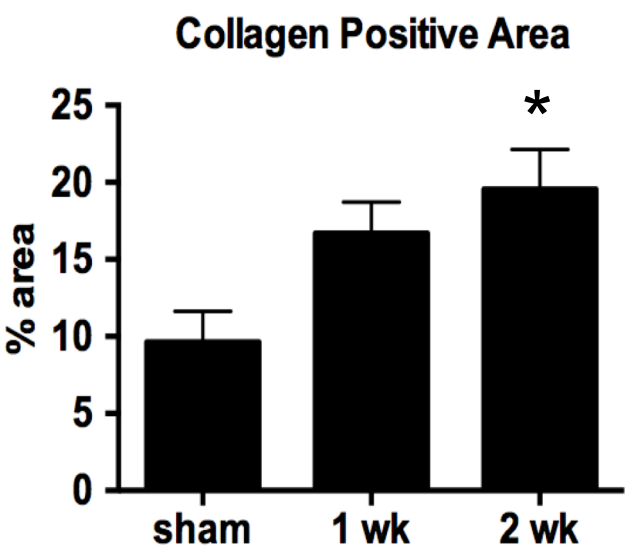


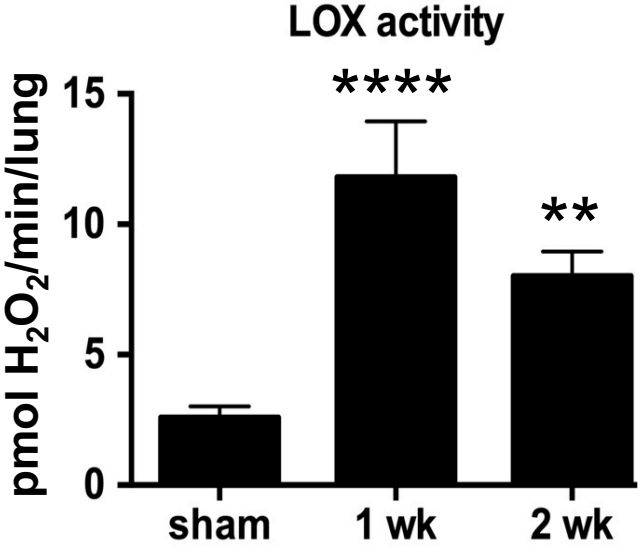
Gd-Hyd imaging analysis correlates with histology and ex-vivo tissue analyses

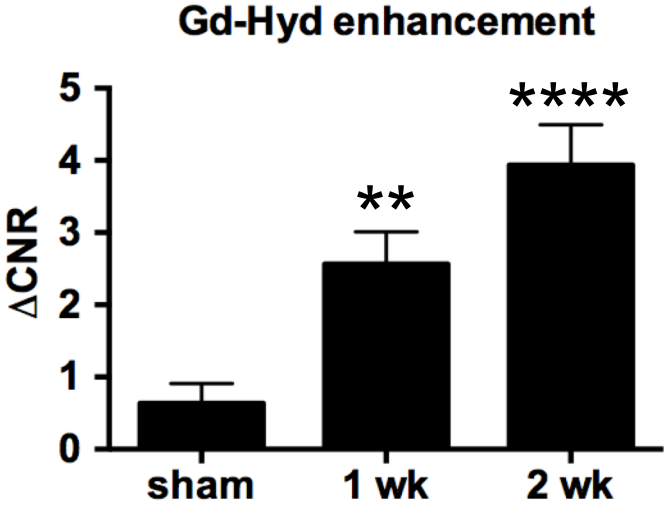






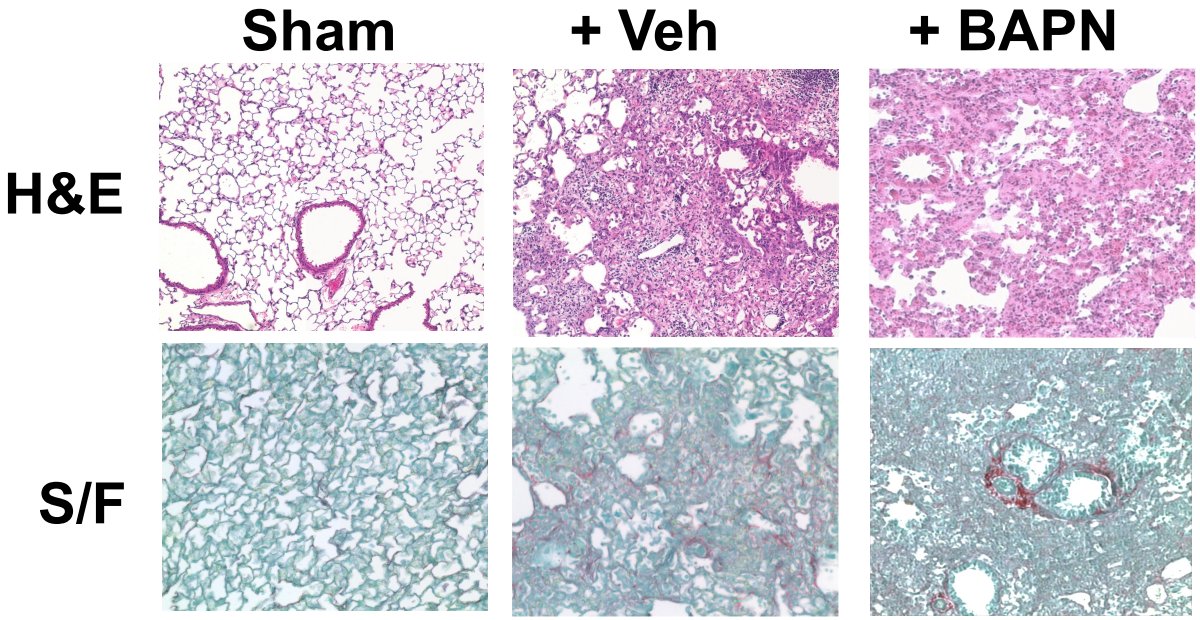


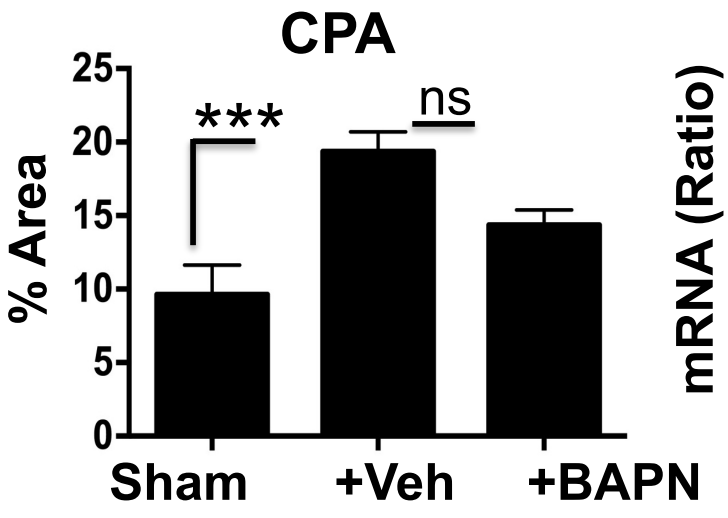


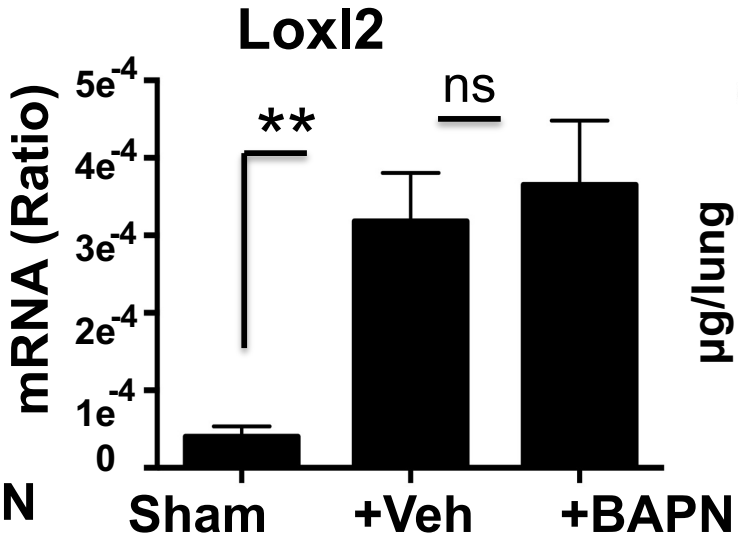


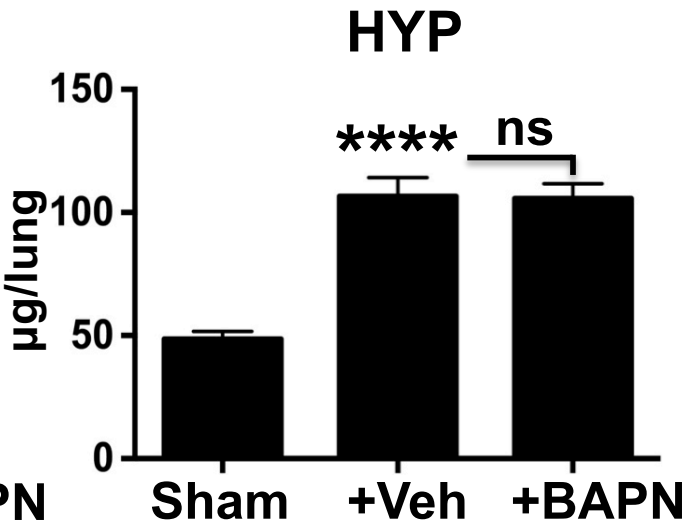
Statistics: * (P≤0.05), ** (P≤0.01) **** (P≤0.0001)

Effect of LOX inhibition (BAPN) on fibrosis









Statistics: * (P≤0.05), ** (P≤0.01) *** (P≤0.001)

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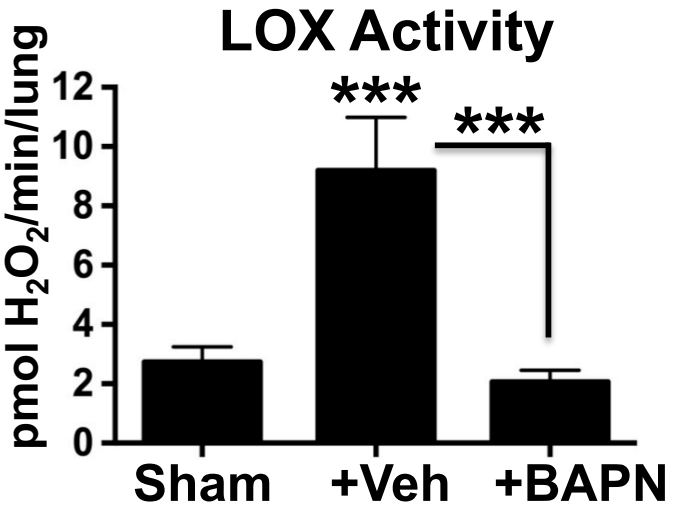
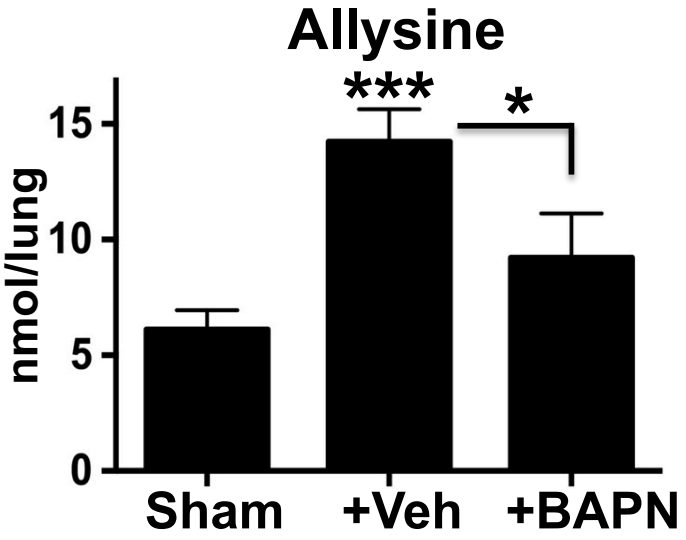
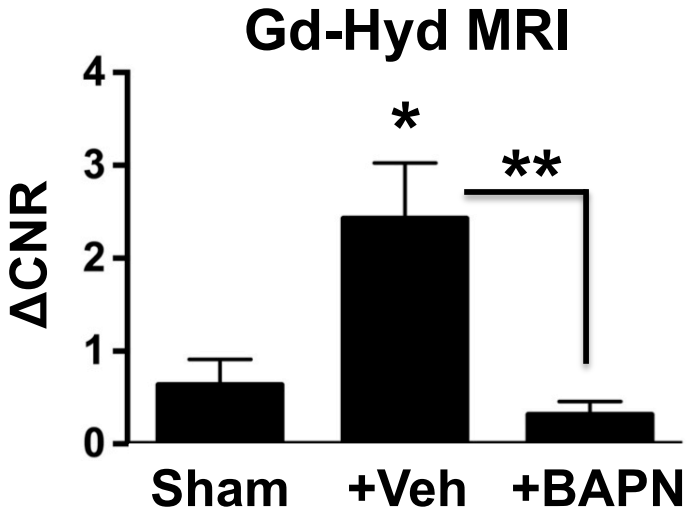
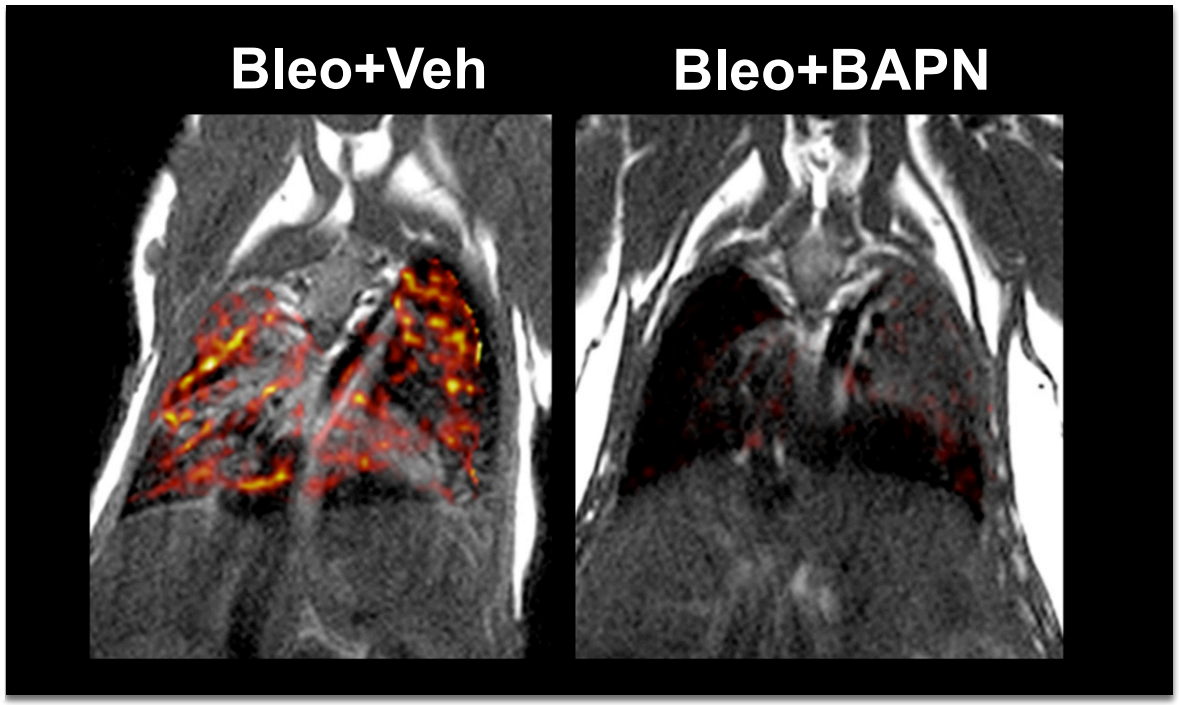
Effect of LOX inhibition (BAPN) on Gd-Hyd imaging



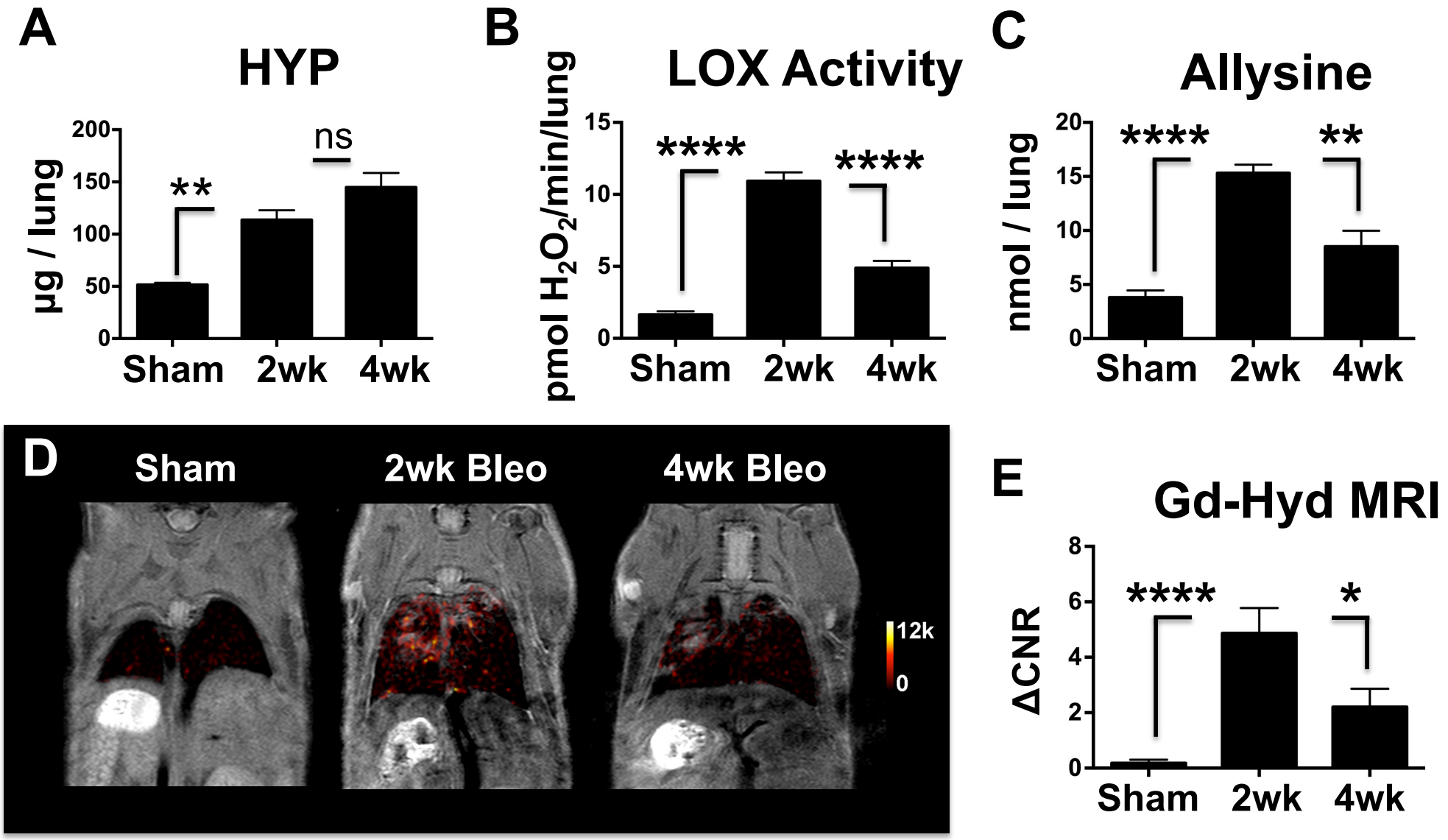






Statistics: * (P≤0.05), ** (P≤0.01) *** (P≤0.001)

Can we distinguish fibrogenesis from stable scar?



Statistics: * (P≤0.05), ** (P≤0.01) *** (P≤0.001)

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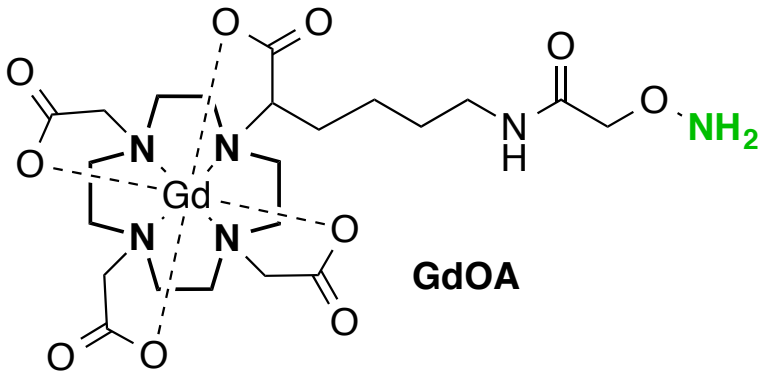
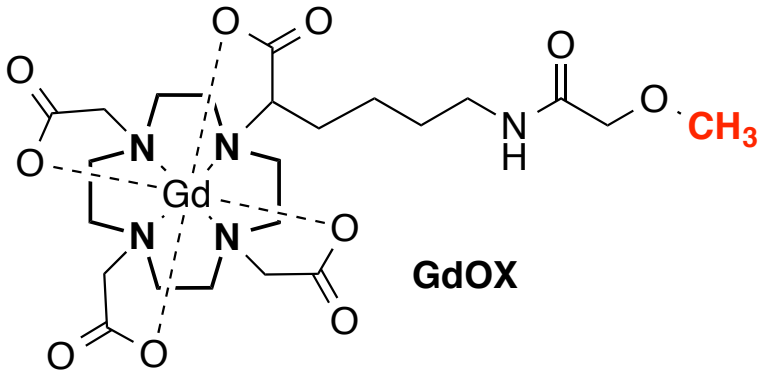
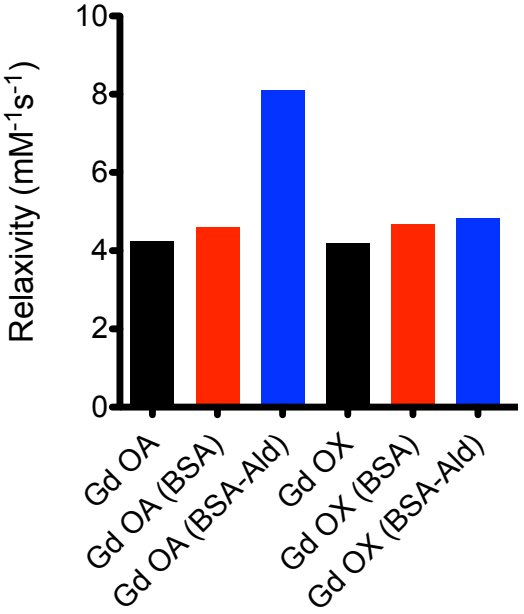
Gd-OA: Improved allysine targeting



GdOA

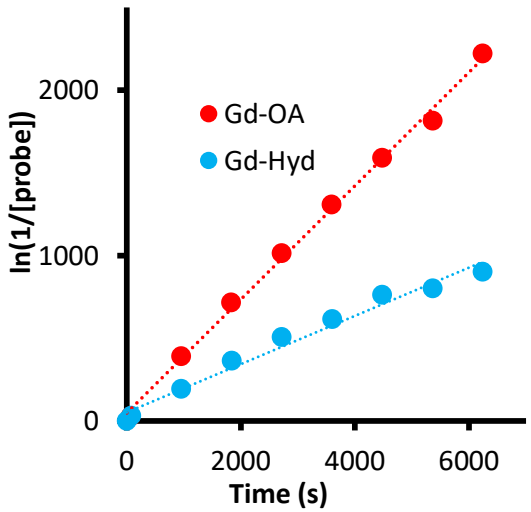


GdOX

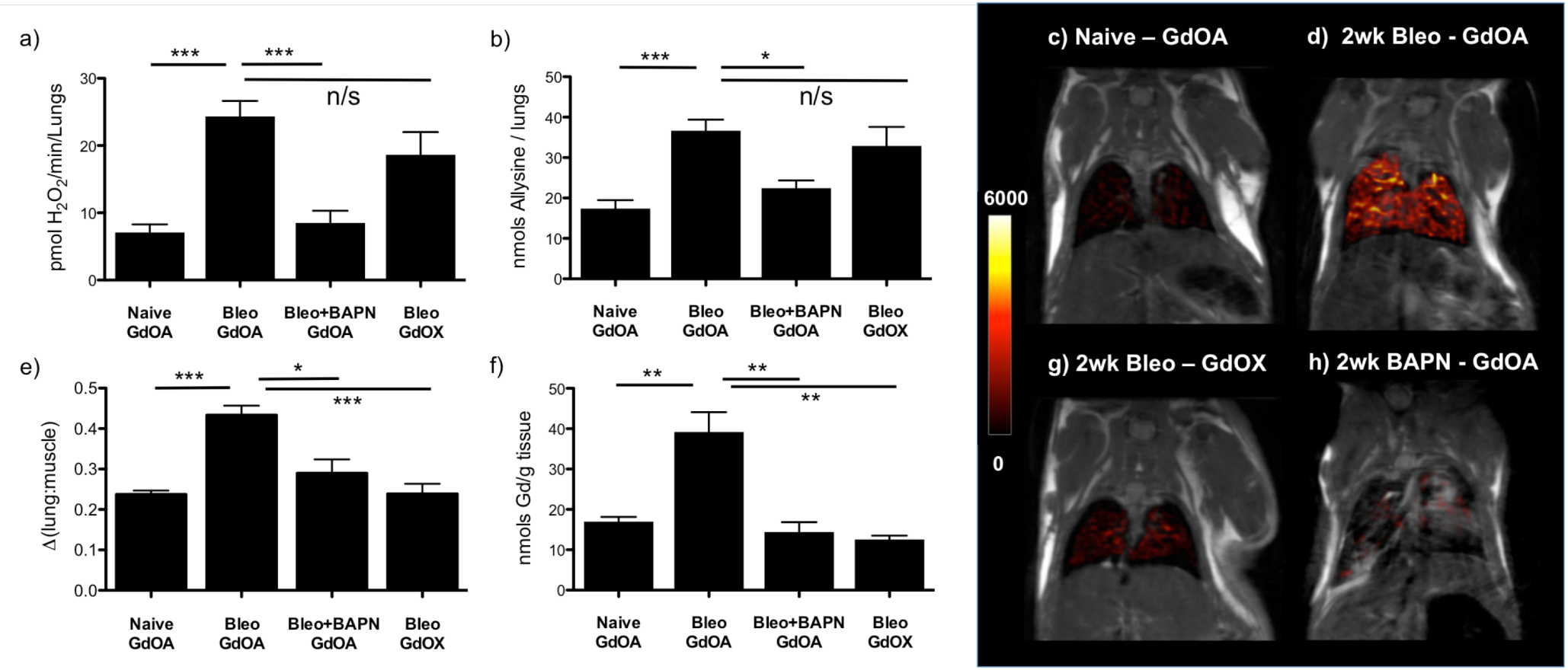


Gd-OA:

- 2 fold higher affinity than Gd-Hyd
- 2 fold higher reactivity to aldehydes than Gd-Hyd
- 2 fold higher observed relaxivity with oxidized BSA



Improved in vivo performance



CNR change with Gd-OA is 2-fold higher
in diseased animals than with Gd-Hyd

Waghorn Angew Chem Int Ed. 2017;56:9825.

Kidney fibrosis

- Kidney fibrosis occurs in many acute and chronic kidney diseases, and also in transplant
- Fibrosis correlates with poor outcome but no good way to noninvasively assess fibrosis
- Collagen COL4A3 knockout: a mouse model for autosomal Alport syndrome
- Mice develop a progressive glomerulonephritis with microhematuria and proteinuria, consistent with the human disease
- Use Gd-OA to identify fibrosis in these mice
- Image before and 4 hours after Gd-OA injection (100 μmol/kg) at 9.4T; measure T1 and correlate with histology, hydroxyproline, LOX activity, and renal function tests

Collaborators and Acknowledgements

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Support

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