SFRR-E 2021 EUROPE

BELGRADE SERBIA 15-18 JUNE 2021

PRESENTED VIRTUALLY

Slide 1

Kinetic data for the reactions of alpha,beta-unsaturated aldehydes shed light on their molecular targets and biological effects

Max B. Sauerland, Luke F. Gamon, Ralf Mertes, Chiara Morozzi, Aimee Eggler, Michael J. Davies

Department of Biomedical Sciences,
University of Copenhagen
Villanova University (USA)

maxsauerland@sund.ku.dk
@MaxSauerland (Twitter)

novo nordisk fonden

Department of Biomedical Sciences

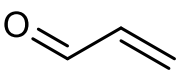
SFRR-E 2021 EUROPE
BELGRADE SERBIA 15-18 JUNE 2021
PRESENTED VIRTUALLY

Abstract

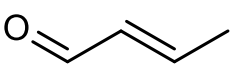
A wide range of alpha, beta-unsaturated carbonyls (ABuCs) are encountered in everyday life. The toxic molecule acrolein is the smallest ABuC family member, which is present in cigarette smoke, fried foods or automobile exhausts. Peroxidation of polyunsaturated fatty acids also yields acrolein among other ABuCs such as crotonaldehyde, 4-hydroxynonenal and 4-hydroxypentenal. The latter ABuCs have also been proposed to have a signaling action rather than a toxic one. ABuCs have also been utilized as treatments for diseases, with dimethylfumarate used to treat multiple sclerosis and psoriasis, and itaconate showing promise as an anti-inflammatory agent. ABuCs act as soft electrophiles and react with biological nucleophiles via Michael addition. Cysteine residues are major targets since they are relatively abundant and kinetically-favored over other targets (e.g. DNA bases, lysine and histidine residues on proteins). However, the kinetics and selectivity of ABuCs–cysteine reactions are incompletely understood. We therefore aimed to determine kinetic data (rate constants) for these reactions and their downstream effects. The rate constants for addition of GSH to acrolein, crotonaldehyde, dimethylfumarate, cyclohex-1-en-2-one and cyclopent-1-en-2-one are shown to vary by a factor of 350 (rate constants, k, 0.5 – 186 M-1 s-1) indicating that the ABuC structure is a determining factor for reactivity, with acrolein being the most reactive. We also show that the microenvironment of the cysteine residue, and its pKa value, have an impact on the reactivity. Protein incorporated cysteine react up to 30 times faster than free cysteine and GSH. The toxic species acrolein is highly reactive, and reacts in an unspecific manner. Dimethylfumarate on the other hand reacts more slowly and is much more specific. Enzymatic assays show that these Michael addition reactions can inhibit enzyme activity, highlighting the functional consequences of ABuC reactivity. These data can help explain why acrolein is toxic, while dimethylfumarate has beneficial biological effects.

Background

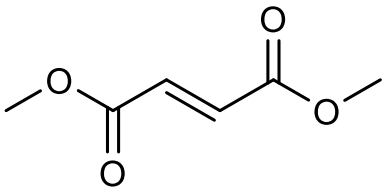
- **Alpha, beta-unsaturated carbonyls (ABuCs) contain an electrophilic beta carbon atom which reacts with nucleophiles to form a Michael adducts.**
- **ABuCs differ in their biological effects:**
  - **acrolein is toxic**
  - **crotonaldehyde is a suggested signaling molecule**
  - **dimethylfumarate (DMFU) is an FDA-approved drug for multiple sclerosis and psoriasis.**
- **Different molecular targets could rationalize those distinct effects.**
- **The specificity of ABuC-nucleophile interactions are not well understood limiting the development of ABuC-based drugs.**



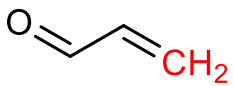
acrolein



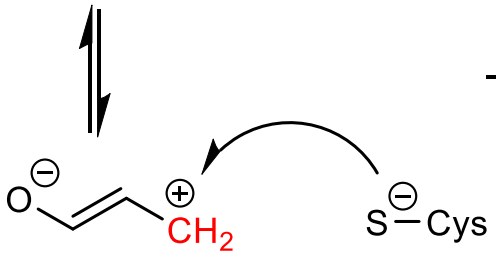
crotonaldehyde

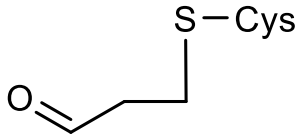


dimethylfumarate



ABuC





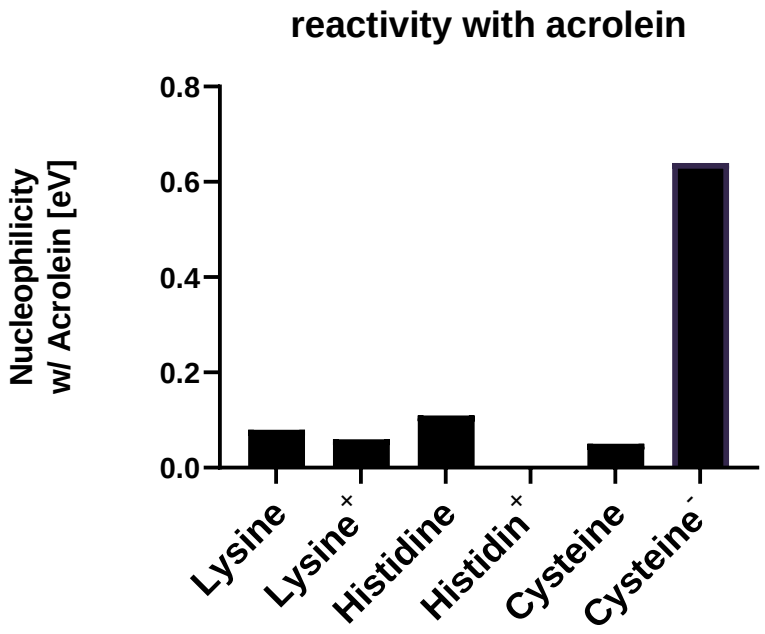
Michael adduct
at cys residue

Hypothesis and Aims

- **Negatively-charged (ionized) Cys residues are the most reactive nucleophiles for ABuCs**
  - **→ Proteins with low pKa cysteine residues have been predicted to be major targets**
- **Many different protein targets have since been identified**

Hypothesis: **Not all observable protein targets have biological relevance, with those that are kinetically most reactive likely to be important**

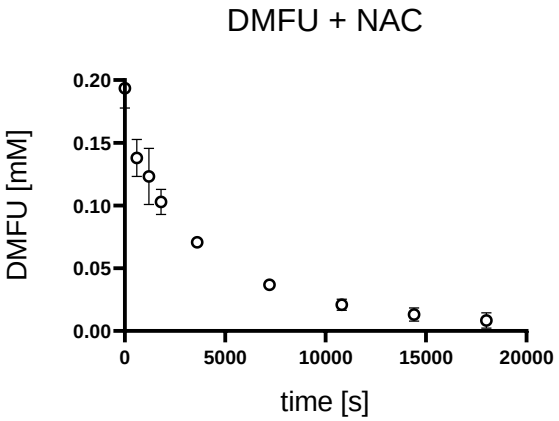
Aims: **Characterize acrolein, crotonaldehyde, DMFU and identify their reaction partners**

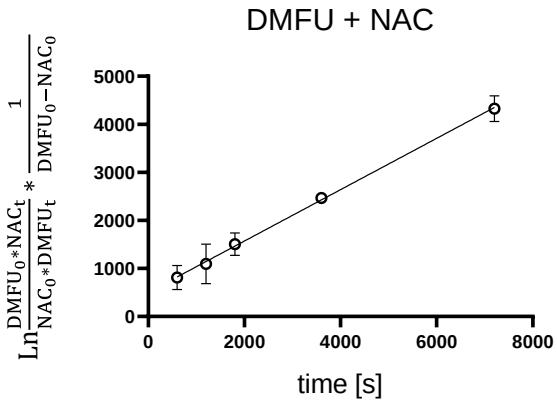


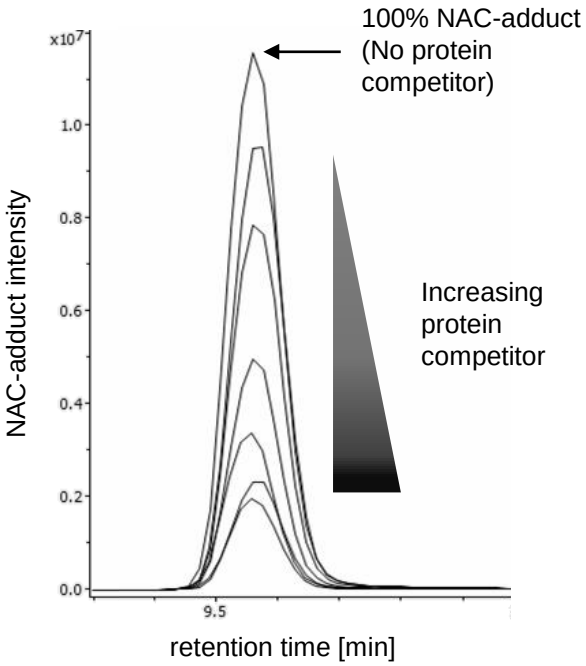
Identified Acrolein Targets
N-acetylcysteine (NAC)
Glutathione (GSH)
Bovine serum albumin
GAPDH
Creatine kinase
Papain
Alpha-synuclein
DNA methyl transferase
Gluthathione reductase
Human serum albumin
Lysozyme
Protein disulfide isomerase
Pyruvate dehydrogenase
Thioredoxin
Tyrosine phosphatase 1B
α-Ketoglutarate dehydrogenase

Methods

- **Kinetic data for reaction of a ABuC (here DMFU) with a nucleophile (here N-acetylcysteine [NAC]) was obtained by quantifying the ABuC concentration over time using HPLC detection (left panel).**
- **The second order rate constant was derived from the kinetic plot (middle panel).**
- **Kinetic data for reaction of ABuC with proteins was obtained using competition kinetics, as the concentration of the ABuC-NAC adduct decreases with increasing concentration of a competitor protein (right panel). Standard competition kinetic analysis yields the rate constant for reaction with the protein.**

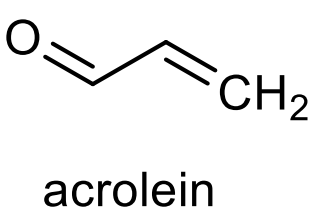




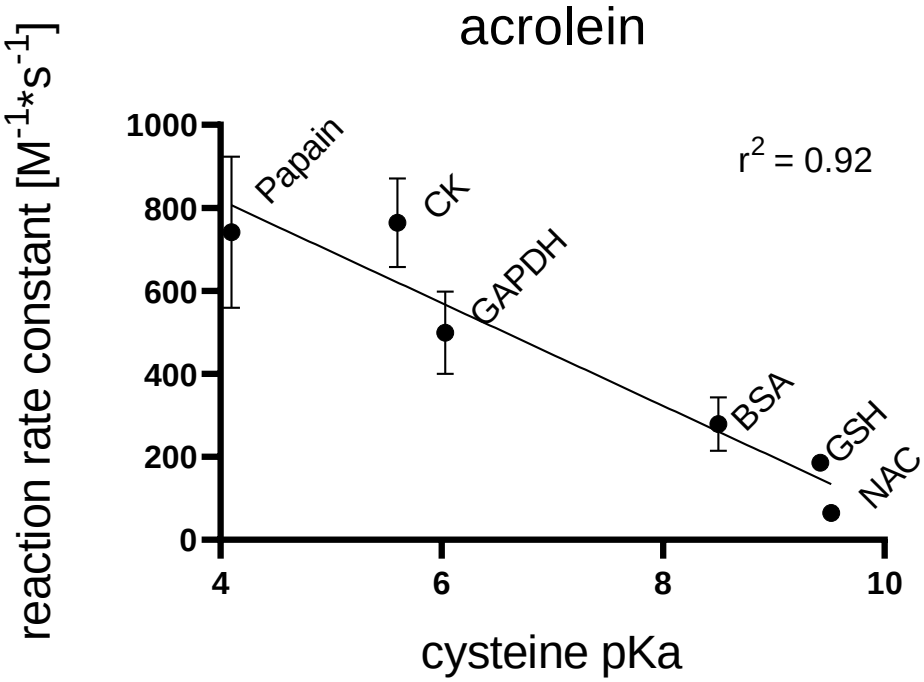


Acrolein reacts unspecific

- **Acrolein (left panel) is a small, uncharged molecule and shows toxic effects *in vivo***
- **The kinetic data show that acrolein has**
  - **High second order rate constants for reaction with Cys residues (*k* 65 - 764 M^{-1} s^{-1})**
  - **A low specificity and is strongly dependent on the Cys pK_a value (right panel)**
- **These kinetic data indicate that acrolein is a non-specific and highly reactive species consistent with its toxic character**

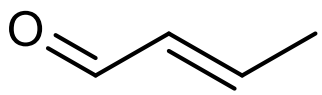


acrolein

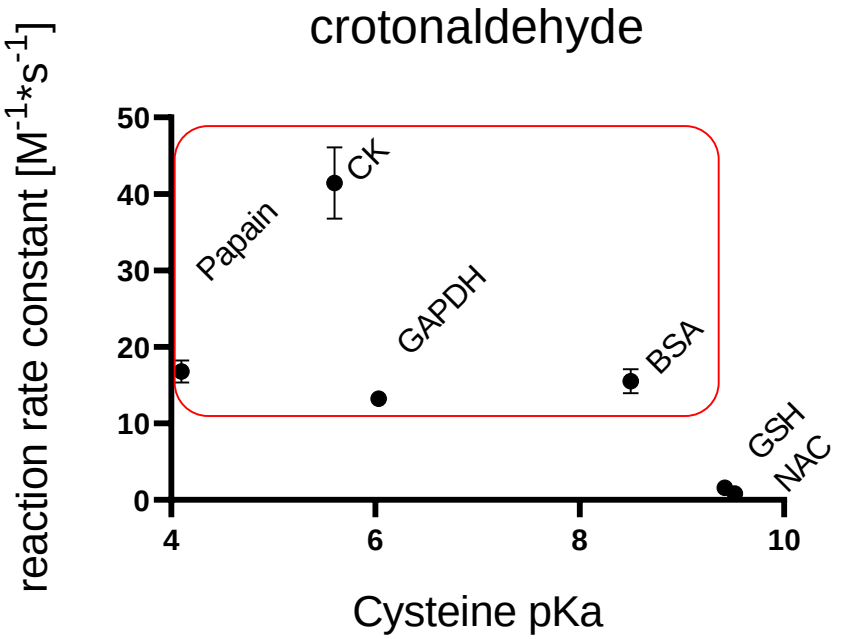


Crotonaldehyde (CA) could act as a signalling molecule

- **CA (left panel) has an additional methyl group when compared to acrolein at the terminal carbon, but**
  - **reacts 20 times slower (*k* 0.81 - 41 M^{-1} s^{-1}) than acrolein**
  - **the reaction rates are only weakly dependent on the pK_a value of the Cys residue**
  - **favors reaction with protein Cys residues over low molecular mass cysteine species**
- **Our data support that crotonaldehyde could be an signaling molecule generated on peroxidation of polyunsaturated fatty acids**

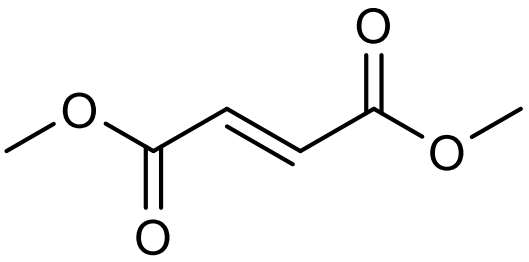


crotonaldehyde

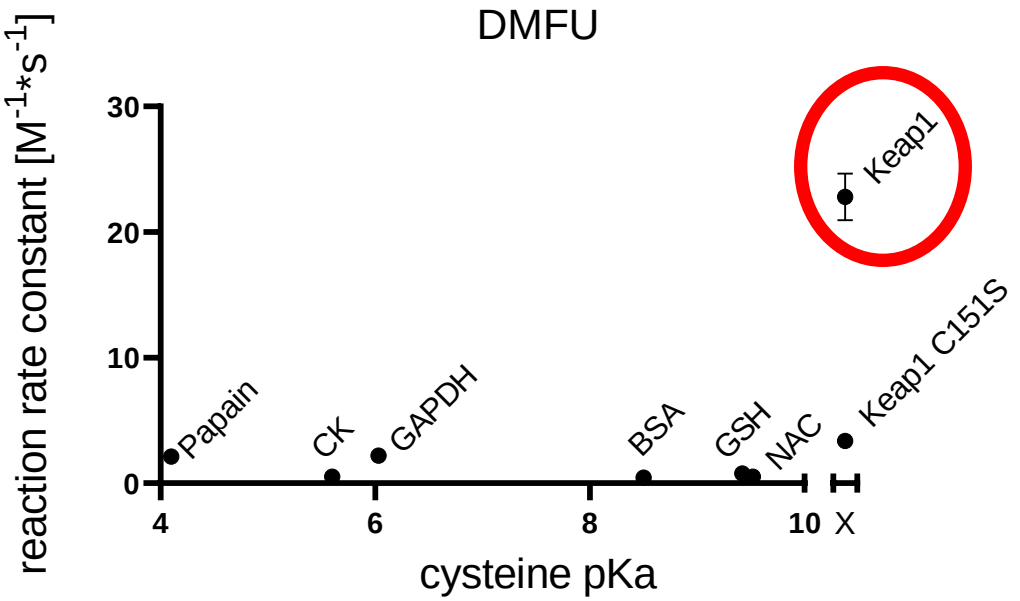


Dimethylfumarate (DMFU) reacts in a highly specific manner

- **DMFU (left panel) is used as a drug and is therefore assumed to react with specific targets**
- **The data support this higher degree of selectivity as:**
  - **it reacts 40 times slower than acrolein (*k* 0.52 - 23 M^{-1} s^{-1})**
  - **the reaction rate constants are not driven by the Cys pKa**
  - **Keap1 is a kinetically favored of DMFU**
  - **Cys151 on Keap1 is a major target, as reaction with the Cys151→Ser mutant is much slower**
- **Inhibition of Keap1 activates the Nrf2 signaling pathway potentially explaining the positive actions of DMFU in multiple sclerosis and psoriasis.**



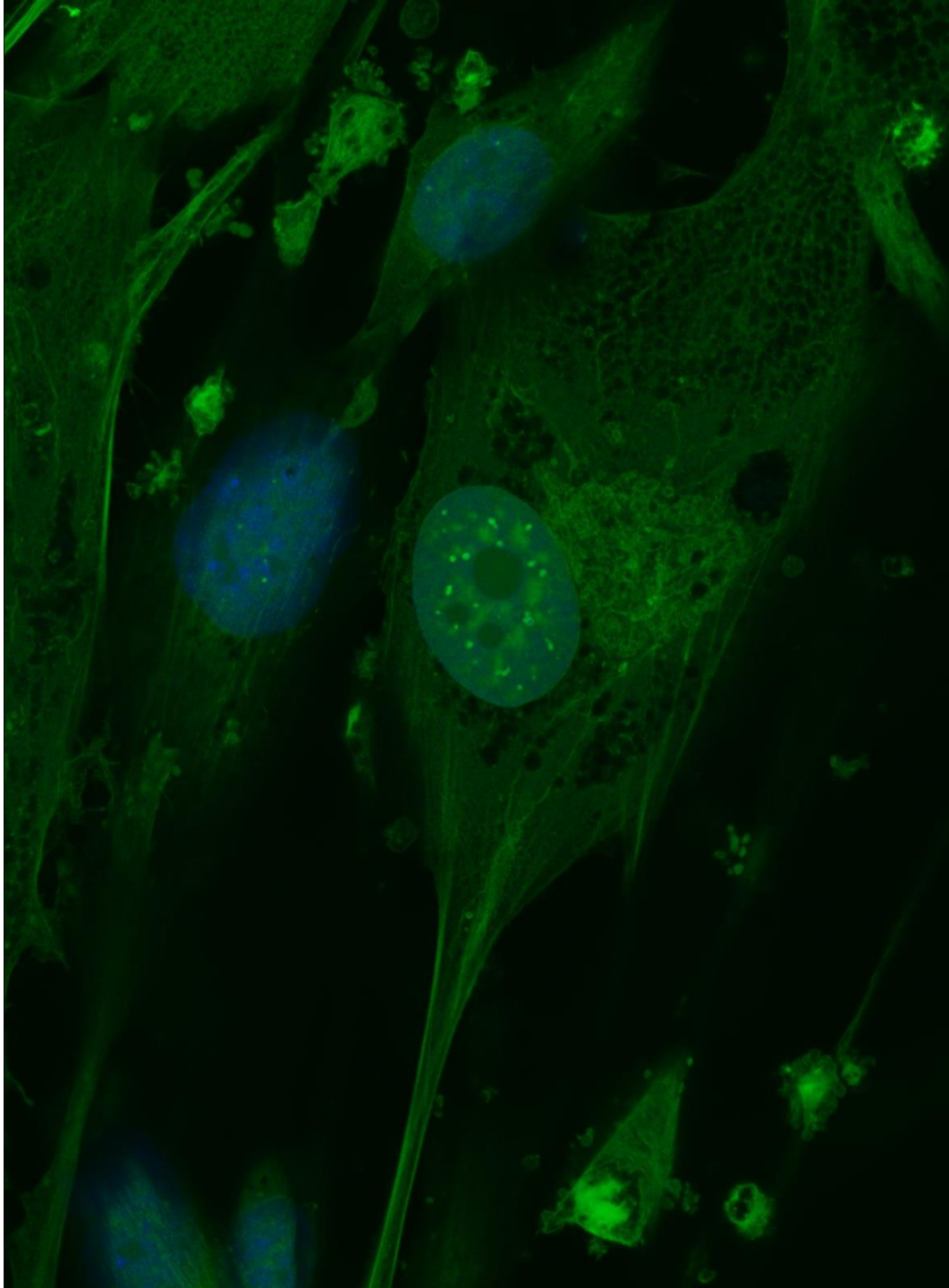
Dimethylfumarate



DMFU has multiple targets

- **DMFU reacts in a specific manner but still with multiple cellular targets**
- **The broad reaction pattern underlines the need to kinetically compare different proteins of interest.**

Human coronary artery smooth muscle cells stained for DMFU modified proteins using a click chemistry probe (green) and cell nuclei (blue)



Conclutions

- **The ABuC structure has a huge impact on its reactivity**
  - **Acrolein reacts ≈ 1500 times faster with creatine kinase than DMFU does**
- **The cellular GSH pool is a competitive target since all ABuCs reacted with GSH**
- **DMFU reacts specifically with Keap1 which explains its positive effects**
- **The kinetic data presented here can help characterize the ABuCs and identify favorable targets. It is therefore a promising approach for future analyses.**

Second order reaction rate constants [M^{-1} s^{-1}]

Peptide/Protein target	Acrolein	Crotonaldehyde	Dimethylfumarate
N-Acetyl-Cysteine	65.0	0.81	0.52
GSH	186.1	1.59	0.79
BSA	279.4	15.5	0.45
GAPDH	499.3	13.2	2.19
Creatine Kinase	764.30	41.4	0.52
Papain	741.4	16.8	2.13
Keap1	-	-	22.8