Stopped Flow Analysis

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CHEM636

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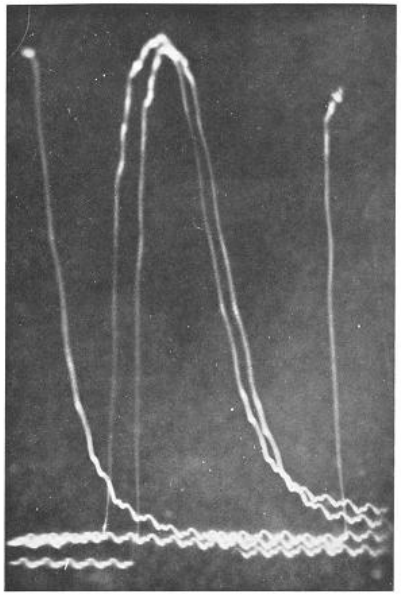
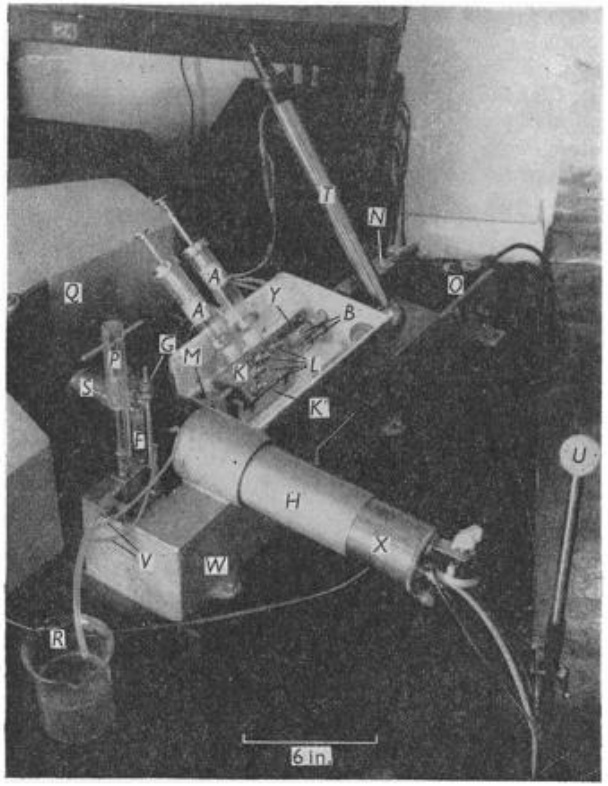
History

- First described by Chance (1940) and Gibson & Milnes (1964), with a view to probing enzyme kinetics
- Oscilloscopes were the first detectors





Chance, B. *J.Franklin Inst.* **1940**, *229*, 613.
Gibson, Q. H.; Milnes, L. *Biochem. J.* **1964**, *91*, 161.

Overview

- **Stopped flow** is a type of flow-injection analysis
- Reactants are rapidly mixed in a **mixing chamber**, and the flow is stopped with the reactant stream in the **flow cell** (detector – usually some form of photometry)
- Allows
  - better measurement sensitivity due to increased residence time
  - reduced fluid dispersion by stopping flow
- **Dead time** – the time between the end of mixing and the start of measurement Typically ms range
  - Measured electronically
  - Shorter is better

Scope

- Small sample volumes (< 0.7 to 500 µL)
- Fast analysis (3 to 60 s)
- $t_{1/2}$ of 1-10 seconds possible
- Sensitivity depends on detector
- Able to tolerate corrosive compounds (e.g. Br_2, H_2SO_4)
- Many instruments commercially available

Other developments

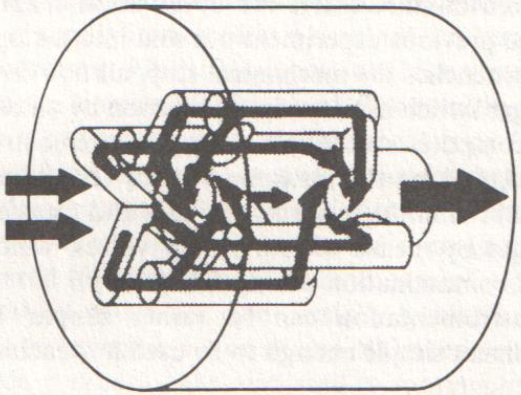
- Wide temperature ranges (e.g. +100 to -100 °C)
- High pressure (2 kbar)
  - measurements of activation volume
- Stopped flow with fast scan spectroscopy or with a temperature jump
  - determine transient reaction intermediates

Gomez-Hens, A.; Perez-Bendito, D. *Anal. Chim. Acta* **1991**, *242*, 147.

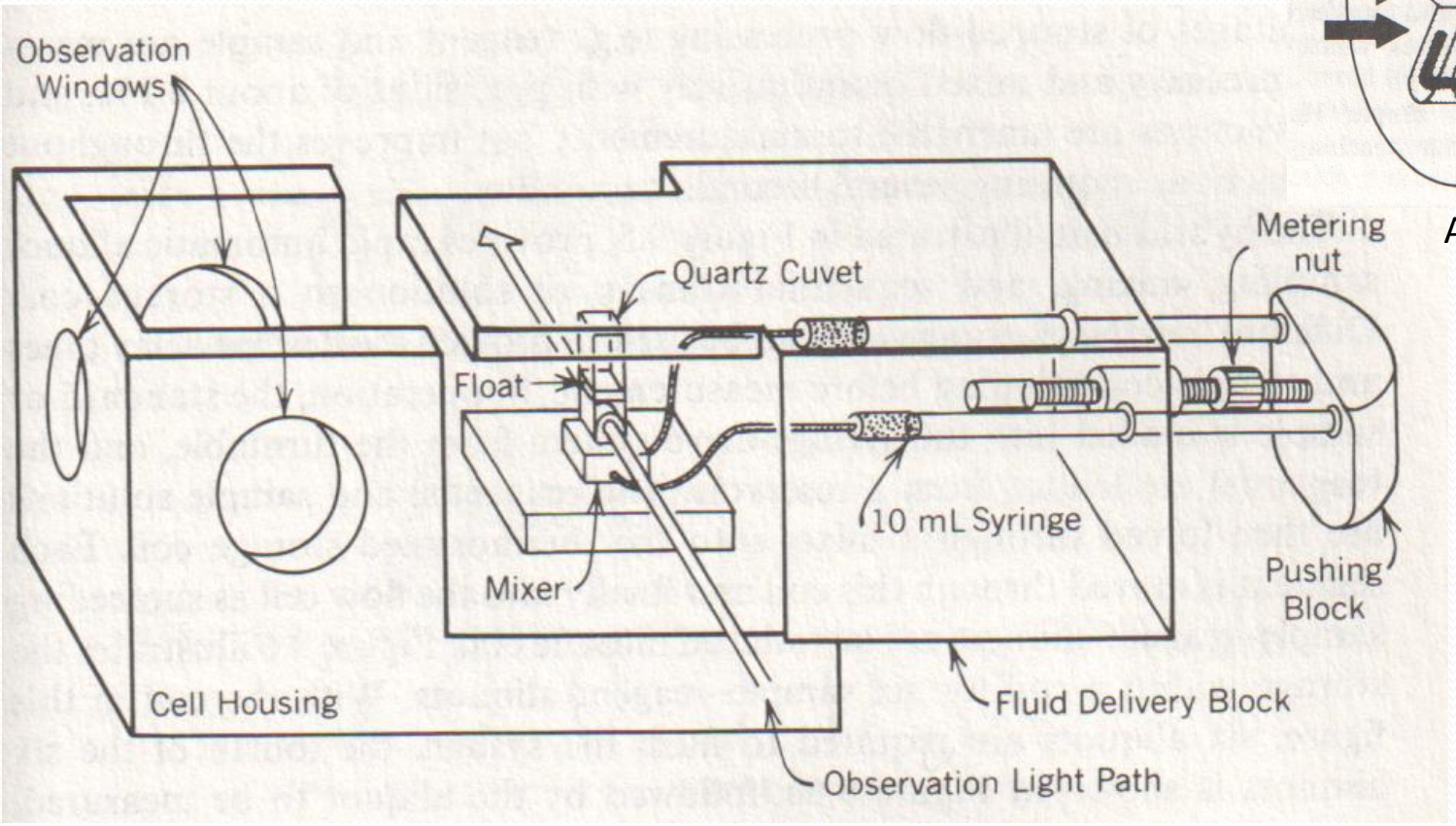
Applications

- Enzymes
- Protein folding
- Redox reactions
- Coordination chemistry
- Catalysis (e.g. polymerization reactions)

Typical Instrument

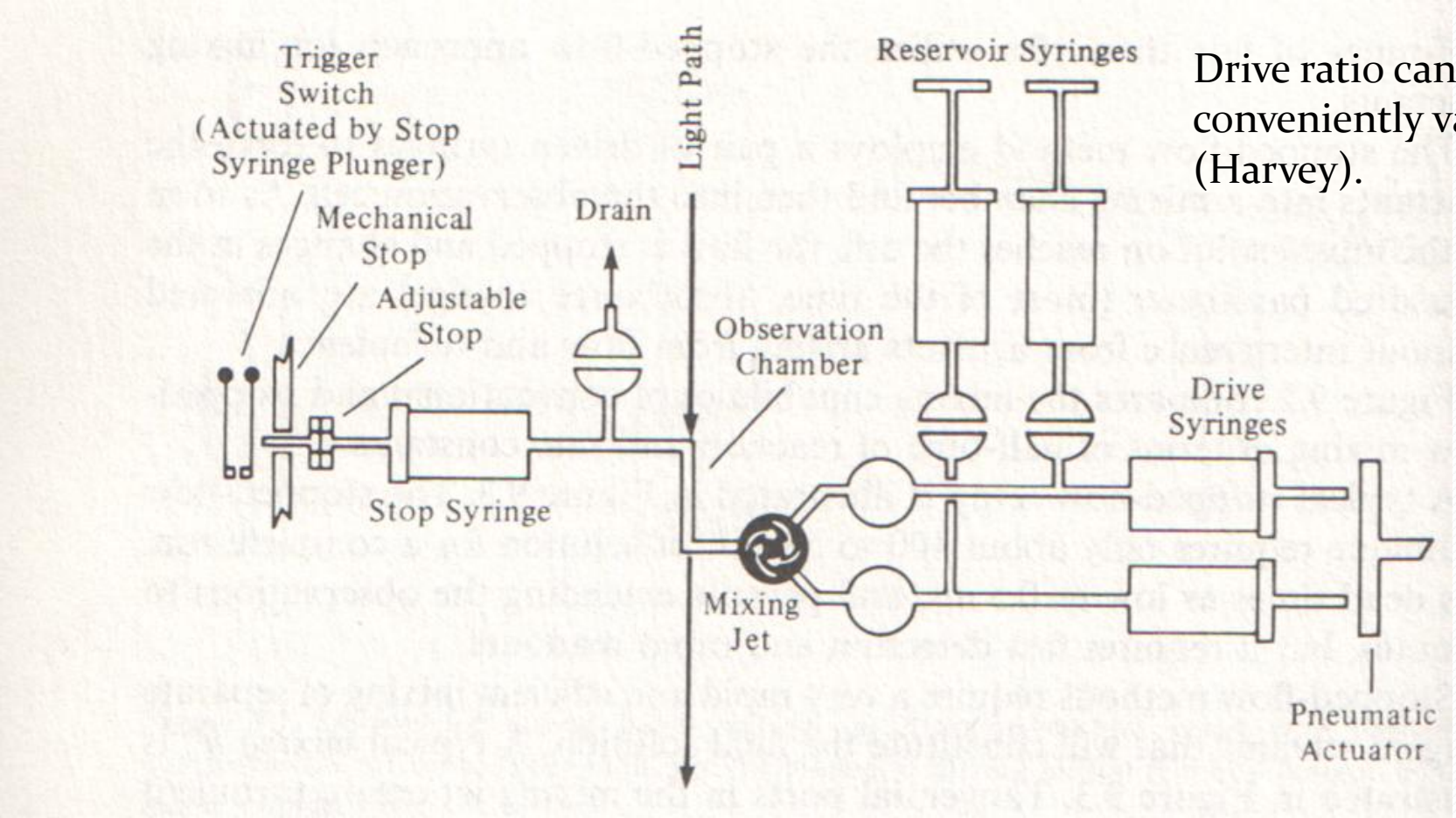


An example of a mixer



Mottola, H. A. *Kinetic aspects of analytical chemistry*; Wiley: New York, 1988; pp 172-178.

Schematics



Drive ratio can be conveniently varied (Harvey).

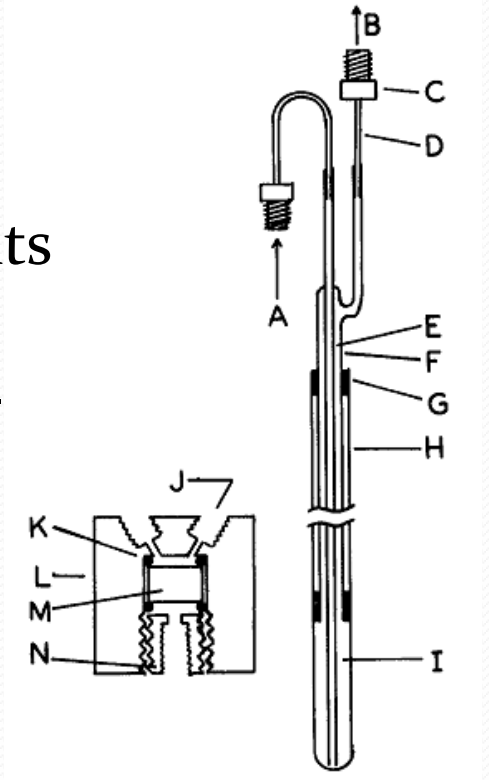
Mottola, H. A. *Kinetic aspects of analytical chemistry*; Wiley: New York, 1988; pp 172-178.
Harvey, R. A.; Borcherdt, W. O. *Anal. Chem.* **1972**, *44*, 1926.

Detectors

- Usually photometric detectors
  - **UV/Vis** & IR
  - **Fluorescence** (more sensitive)
  - Chemiluminescence
  - Circular dichroism (good for protein work)
  - Refractive index
  - NMR* (e.g. ^{1}H, ^{19}F)
  - EPR
  - Voltammetry
  - Electrical conductivity

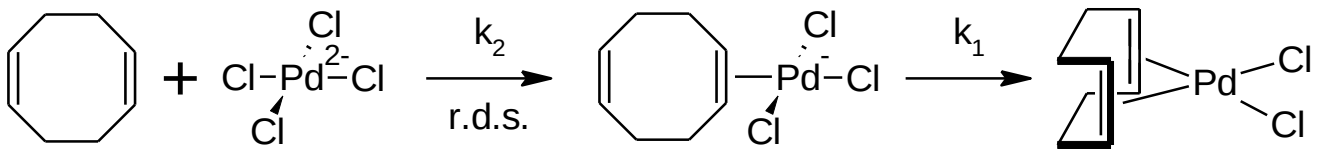
Stopped Flow NMR

- NMR is the most information-rich type of spectroscopy
- Hand-mixing of reagents – useful to get data points within tens of seconds
- Stopped flow is useful to get data points within 2-10 s
- Main delay in this technique is the spin-lattice relaxation time, T_1
  - Reduced by addition of a relaxation agent, e.g. $Cr(acac)_3$, but causes line broadening
- Apparatus not commercially available yet



Christianson, M. D.; Tan, E. H. P.; Landis, C. R. *J. Am. Chem. Soc.* **2010**, *132*, 11461.
Green, D. B.; Lane, J.; Wing, R. *Appl. Spectrosc.* **1987**, *41*, 847.

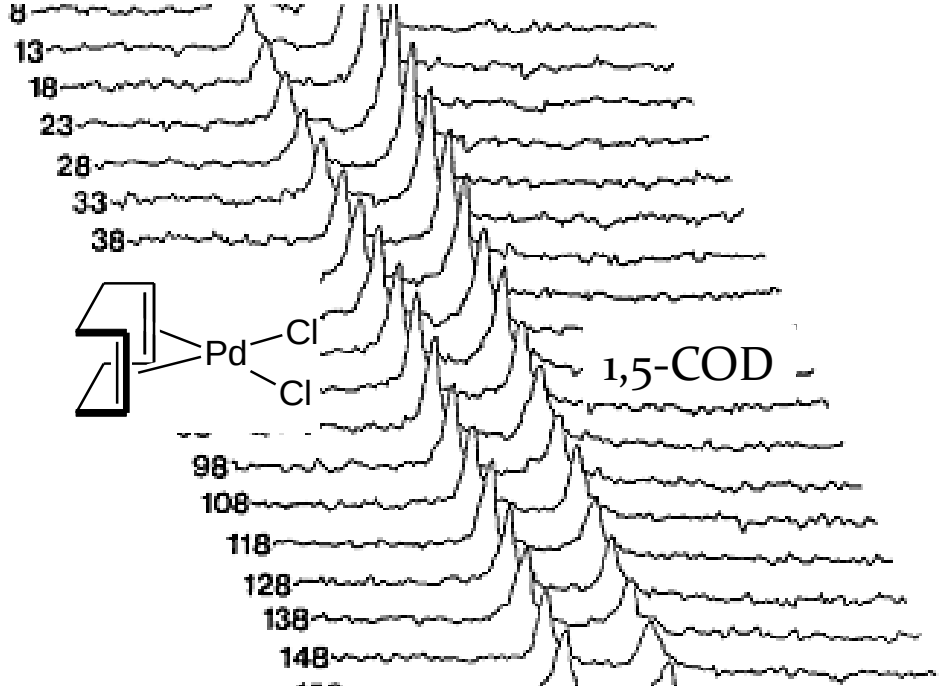
Probing Ligand Substitutions



- 200 ms dead time
- No relaxation agent needed
- Calculated values agree with literature:

$$k_2 = 1.2 \times 10^{-2} M^{-1} s^{-1}$$

$$t_{\frac{1}{2}} = 60\ s$$

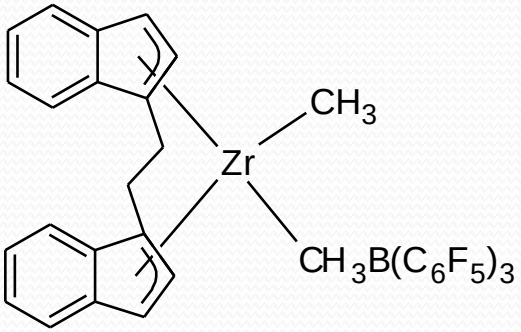


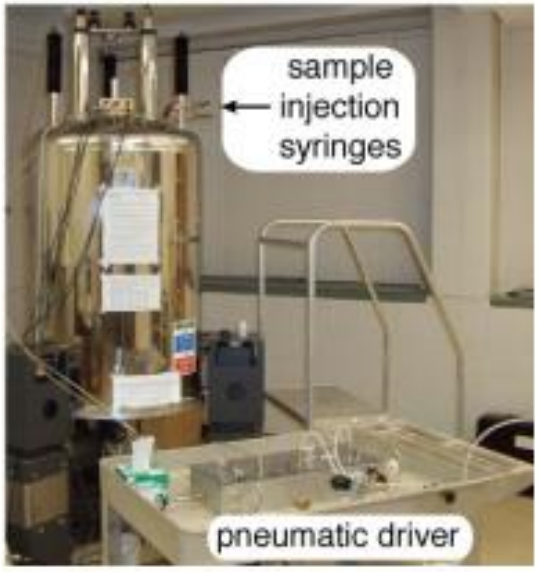
Green, D. B.; Lane, J.; Wing, R. *Appl. Spectrosc.* **1987**, *41*, 847.

Further reading: Christianson, M. D.; Landis, C. R. *Concepts Magn. Reson., Part A* **2007**, *30A*, 165.

Studying the Mechanism of Catalytic Polymerization

- Landis *et al.* studied the Zr-catalyzed polymerization of 1-hexene
- Commercial NMR flow probe modified to give mixing chamber & related stopped flow apparatus
- Three reagent concentrations varied: 1-hexene, and 2 catalyst precursors (*rac*-(C_2H_4(1-indenyl)$_2$)$ZrMe_2$ & $B(C_6F_5)_3$)
- Multiple "shots" used to sample the course of each reaction for every set of parameters





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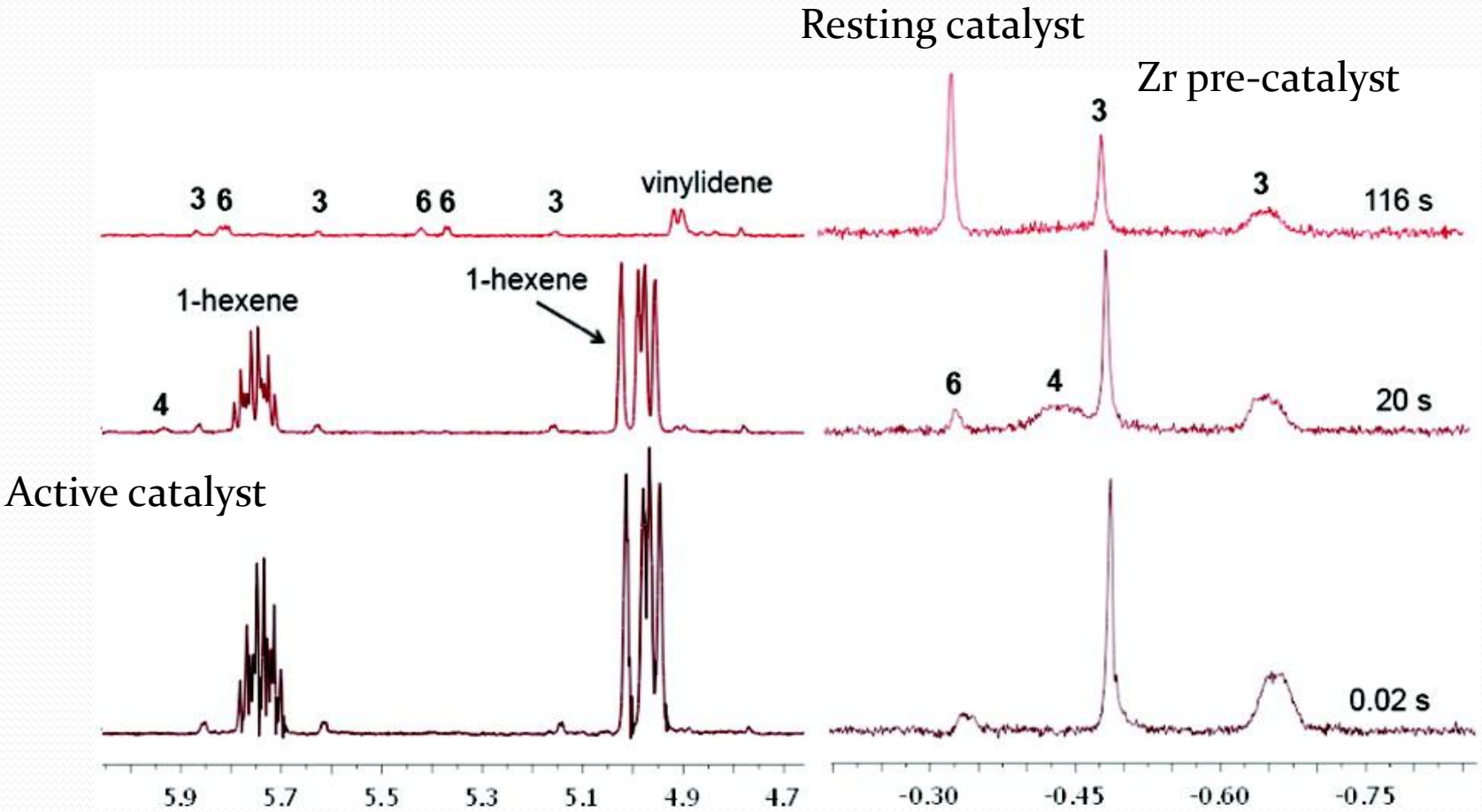
Data



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Analysis

	Initiation	Propagation	Chain Transfer	Re-initiation
entry	**$k_i/M^{-1}s^{-1}$**	**$k_p/M^{-1}s^{-1}$**	**k_t/s^{-1}**	**$k_r/M^{-1}s^{-1}$**
1a	0.224(2)	13.2(1)	0.0317(4)	2.70(7)
2a	0.218(2)	18.6(2)	0.0345(4)	4.12(6)
3a	0.255(1)	18.3(1)	0.0303(3)	2.56(3)
4a	0.222(3)	17.8(2)	0.0264(5)	1.62(5)
5a	0.314(3)	21.8(2)	0.0369(6)	2.33(7)
6a	0.215(2)	15.2(1)	0.0339(4)	1.80(3)
global fit (COPASI)	0.2353(6)	16.83(6)	0.0323(2)	2.06(2)
Rapid Quenched Flow (25 °C)	0.25	8.1	0.0132	$>10k_p$
Rapid Quenched Flow (0 °C)	0.033	2.2	0.00066	$>10k_p$
Kinetic Modelling	0.031	3.7	0.0024	10^3k_p

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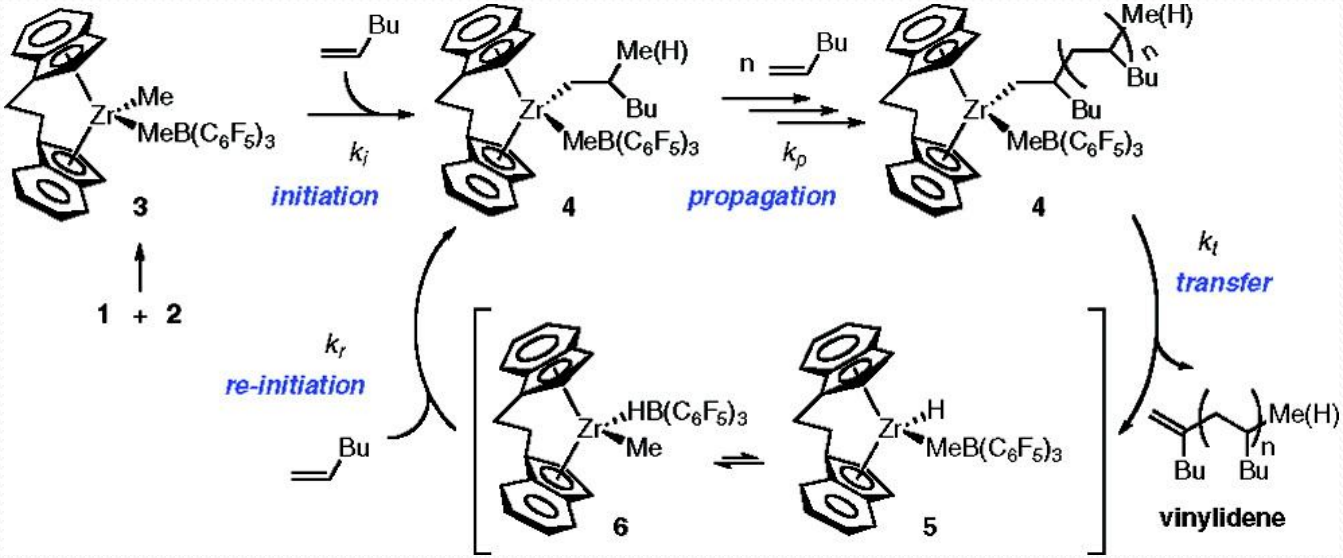
Catalytic Pathway



- Re-initiation of hydridoborate (**6**), originally thought to be fast, found to be very slow
- 43 % of catalyst (**3**) inactive

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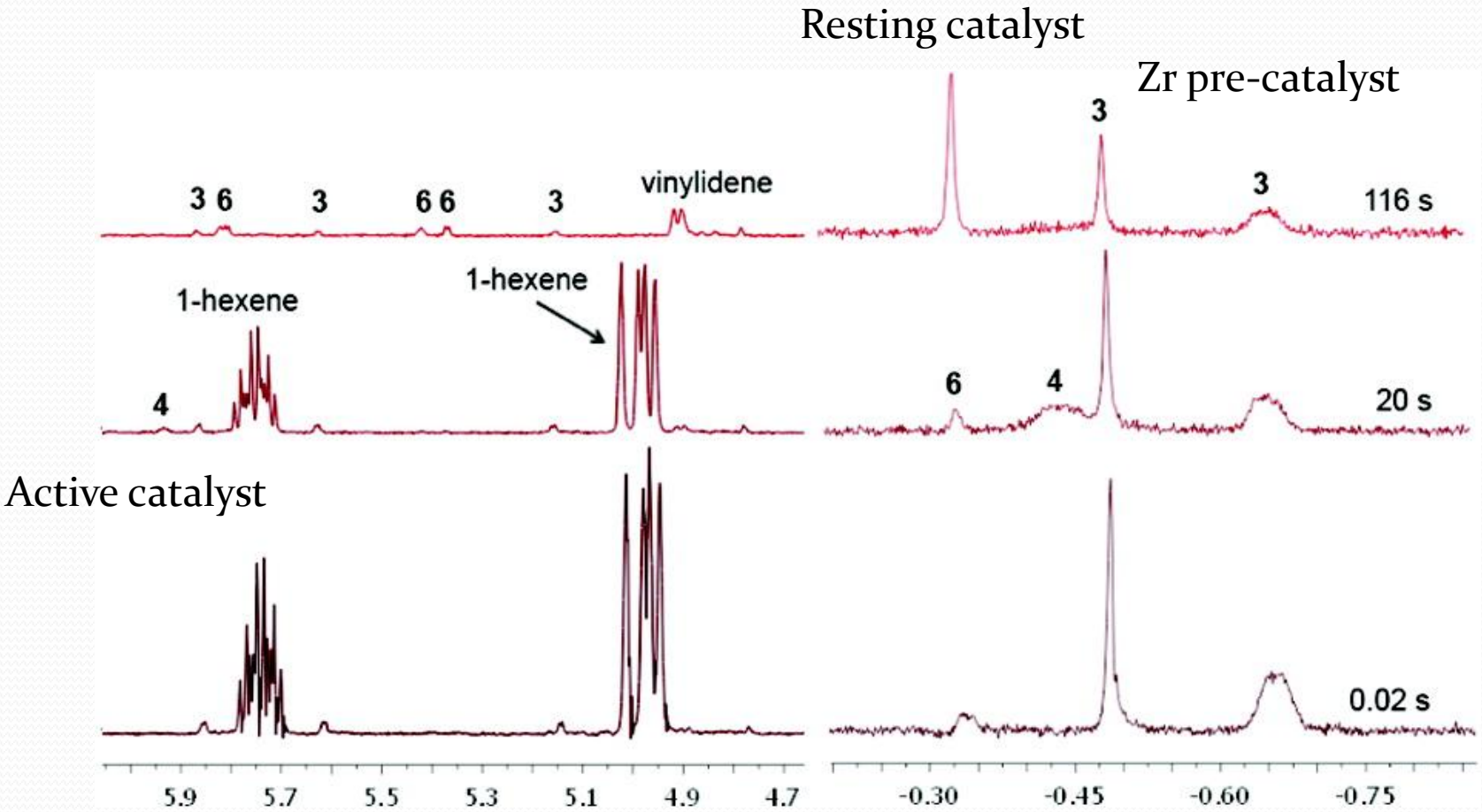
Data



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Questions