ASSIGNMENT (CLASS - 24.03.2020)
BIOMOLECULAR STRUCTURES

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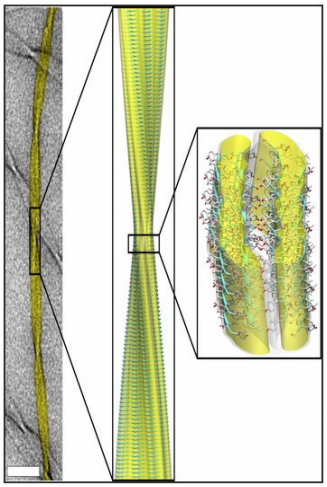
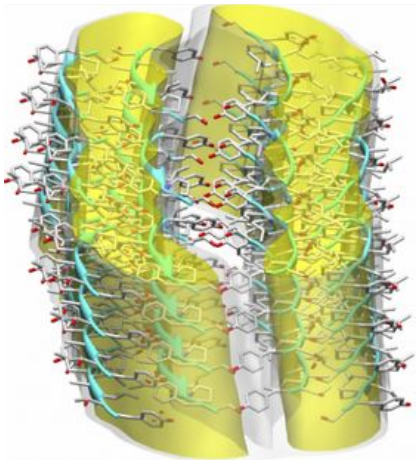
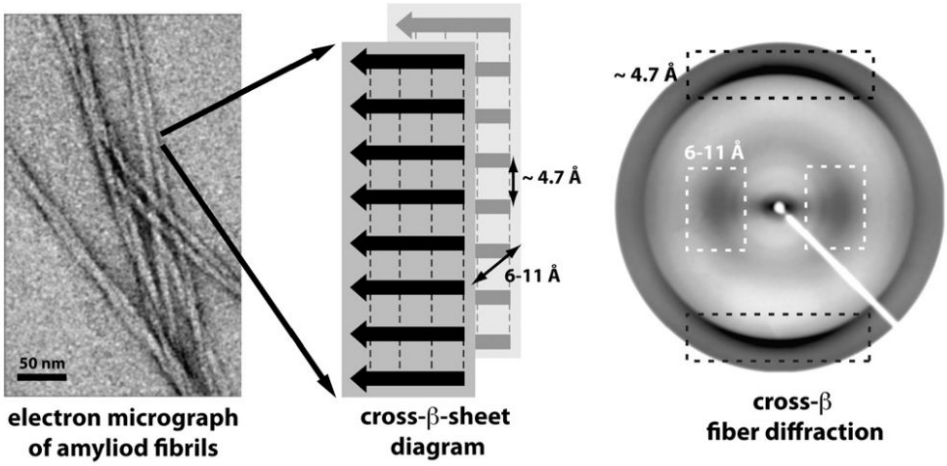
Amyloid Fibrils Structure



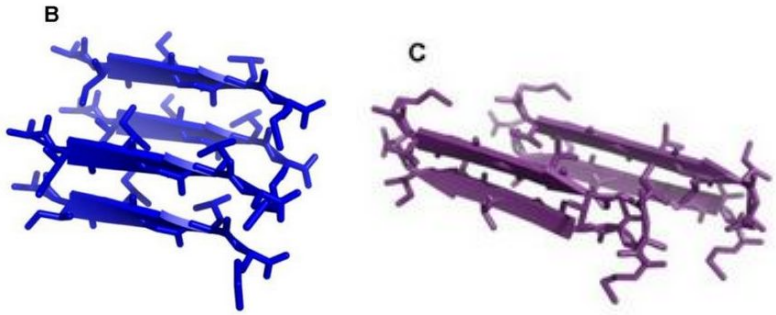


Amyloid fibrils are composed of long filaments that are characterized by an extended beta-sheet secondary structure in which individual β-strands are arranged in an orientation perpendicular to the axis of the fiber. Such a structure is known as cross-β structure. They have single translational and rotational symmetry elements.



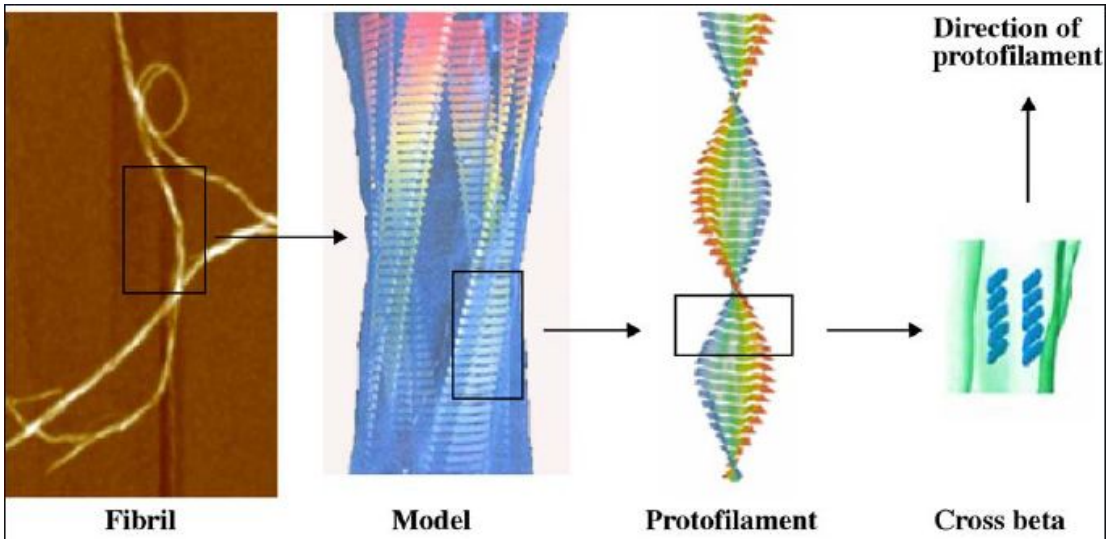
The repeating cross-β sheet motif gives rise to characteristic X-ray fiber diffraction patterns with a meridional reflection at 4.7 Å corresponding to the inter-β strand spacing and an equatorial reflection at 6–11 Å corresponding to the distance between stacked β sheets (one longitudinal and one transverse forming a *cross* pattern). The spacing between the β-sheets depends on the size of the side-chain groups.

Some common characteristics of amyloid fibers are the long, relatively straight and unbranched nature of the fibers; the typical fiber width of 5–15 nm; the periodic twist often observed and many amyloid fibers are made up of the bundling of thinner protofibrils (as seen in the diagrams).



Most amyloids have a parallel β-sheet structure in their cross-β structure (B). (C) is with antiparallel β-sheets. Only a fraction of the polypeptide chain is in a β-strand conformation in the fibrils, the rest form structured or unstructured loops or tails.

The predominant fibrillar structure was composed of two 5nm diameter protofilaments wound in a left-handed direction.



Cryo-electron microscopy and single particle processing of mature amyloid fibrils indicated that a single fibril was composed of 2 to 6 protofilaments wound around a central core, each 2–7 nm in diameter, that interact laterally as flat ribbons that maintain the height of 2–7 nm that of a single protofilament) and are 30 nm wide; more often protofilaments twist around each other to form the typically 5–15 nm wide fibrils.

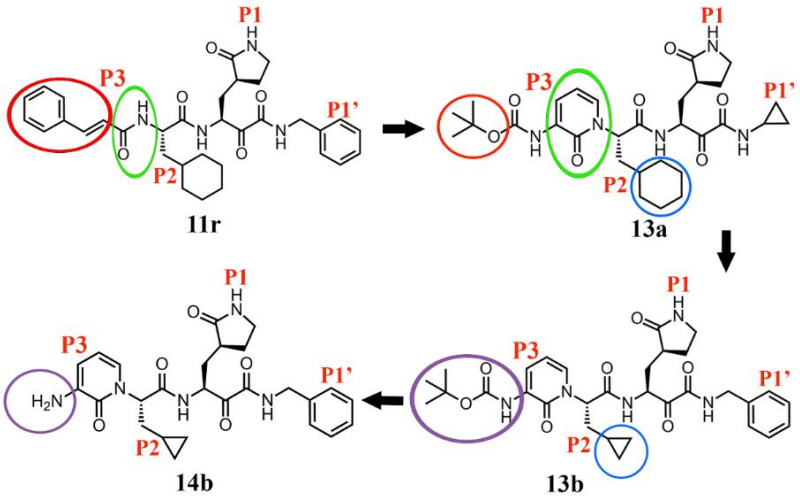
Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors

The main protease (M^{pro}) is an attractive drug target due to its essential role in processing the polyproteins that are translated from the viral RNA - SARS-CoV-2, and inhibiting the activity of this enzyme would block viral replication.

Drug used

The drug of choice is α-ketoamides as they are broad-spectrum inhibitors of the main proteases of betacoronaviruses, alphacoronaviruses and the 3C proteases of enteroviruses. Their warhead can interact with the catalytic center of the target proteases through two hydrogen bonding interactions.

Structural Modifications



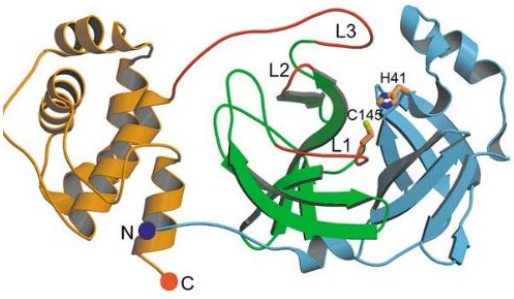
- **11r**:
  - Designed for broad spectrum
  - Low EC_{50} for SARS-CoV and other enteroviruses.
- **13a**:
  - **11r** structurally modified
  - *Pyridone ring added to hide P3 - P2 amide bond (green circles) to prevent cleavage of bond* causing Improved the half-life of the compound in plasma
  - Reduced binding to plasma proteins and Increased the solubility in plasma by *replacing the hydrophobic cinnamoyl moiety by a less hydrophobic Boc group (red circle)*
  - Increased thermodynamic stability
  - Loss of inhibitory activity against the M^{pro} of SARS-CoV-2
- **13b**:
  - **13a** structurally modified
  - Made specific to target protease by *replacing the P2 cyclohexyl moiety with smaller cyclopropyl (blue circle)*
  - Complex has 2 structures now; one in space group C2 (protomers have identical conformation) and another in $P2_12_12_1$ (protomers have different conformations).
  - Stereochemistry is S

    *(Space groups are symmetry groups of a configuration in space usually 3D)
- **14b**:
  - **13b** structurally modified

- *Hydrophobic and bulky Boc group (tert-butyloxycarbonyl) replaced with less bulkier group (purple circle)* making the drug almost inactive which implies the Boc group is necessary to cross the cellular membrane and that *a more hydrophobic moiety may be advantageous.*

Structure of SARS-CoV-2 M^{pro}

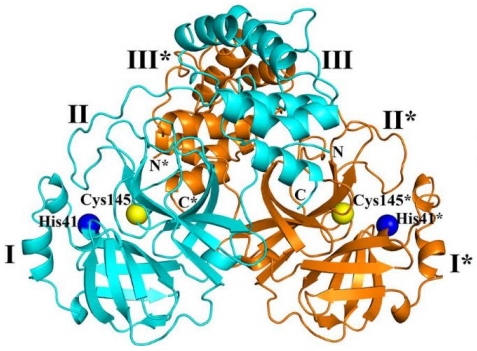
The structure of M^{pro} of SARS-CoV-2 is very similar to that of SARS-CoV. The monomer of SARS-CoV M^{pro} which is inactive because it is the "N-finger" like the N-terminal residue (Ser1 in SARS-CoV) of one monomer that activates the other one.



- Domain I and II - six-stranded antiparallel β-barrels that contain the substrate-binding site between them.
- Domain III - a globular cluster of five helices involved in regulating dimerization of the M^{pro}.

*(Beta barrel: A beta-sheet composed of continuous repeats that twists and coils to form a closed toroidal structure)

SARS-CoV-2 M^{pro} has a tight dimer with the contact interface between domain II of molecule A and the NH_2 - terminal residues of molecule B where both molecules are perpendicular to one another. Dimerization of the enzyme is necessary for catalytic activity, as it helps shape the S1 pocket of the substrate-binding site.



Peptidomimetic Inhibitors and Observations

Peptidomimetic inhibitors (small synthetic protein-like chain designed to mimic a peptide) for **13b**:

- P1: Deeply embedded in S1 pocket; Hydrogen bonded to Phe^{140}, Glu^{166} and His^{163}.
- P2: Fits into a S2 subsite (shrunk for **13b** as compared to **13a**); Hydrogen bonded Glu^{166} .
- P3: Can be bulkier than pyridone (Extra space occupied by DMSO or water); Hydrogen bonded to Glu^{166}.

Assessment of the absorption - distribution - metabolism - excretion (ADME) properties

13a and **13b** when injected subcutaneously did not remain in plasma for long but excreted via urine for a long time. **13a** remained in lung tissues even after 24 hours suggesting that it was mainly distributed to tissue. **13b** showed a less rapid clearance compared to **13a** and was more effective along with high binding to human proteins**.** This lung tropism of **13a** and **13b** is beneficial given that COVID-19 affects the lungs. When inhaled, **13b** showed no adverse effects.