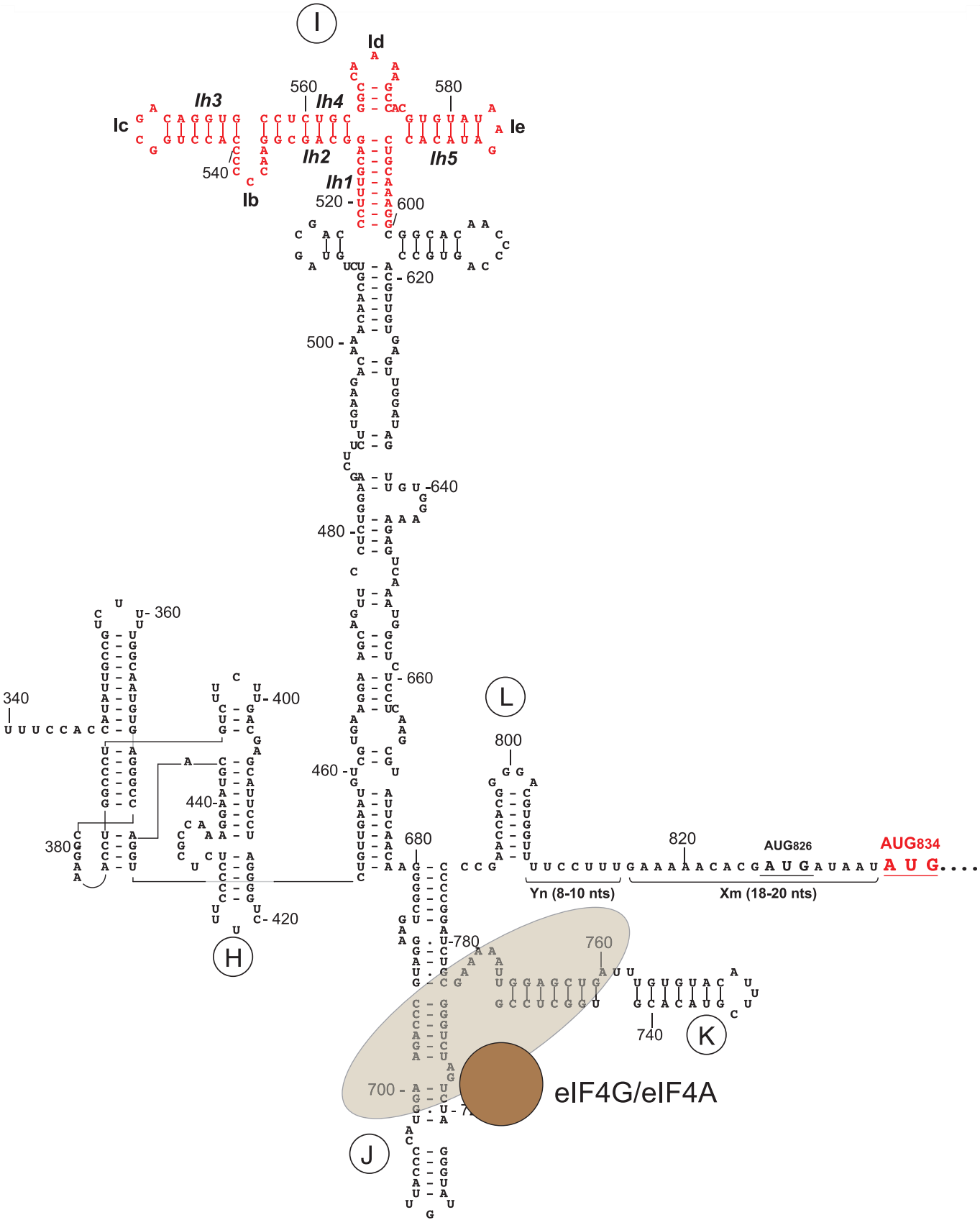


Expanded View Figures

Figure EV1. Secondary structure of the EMCV IRES.

The EMCV IRES secondary structure model featuring individual domains, the region corresponding to cryo-EM density (red), and specific binding of eIF4A/4G to the JK domain.



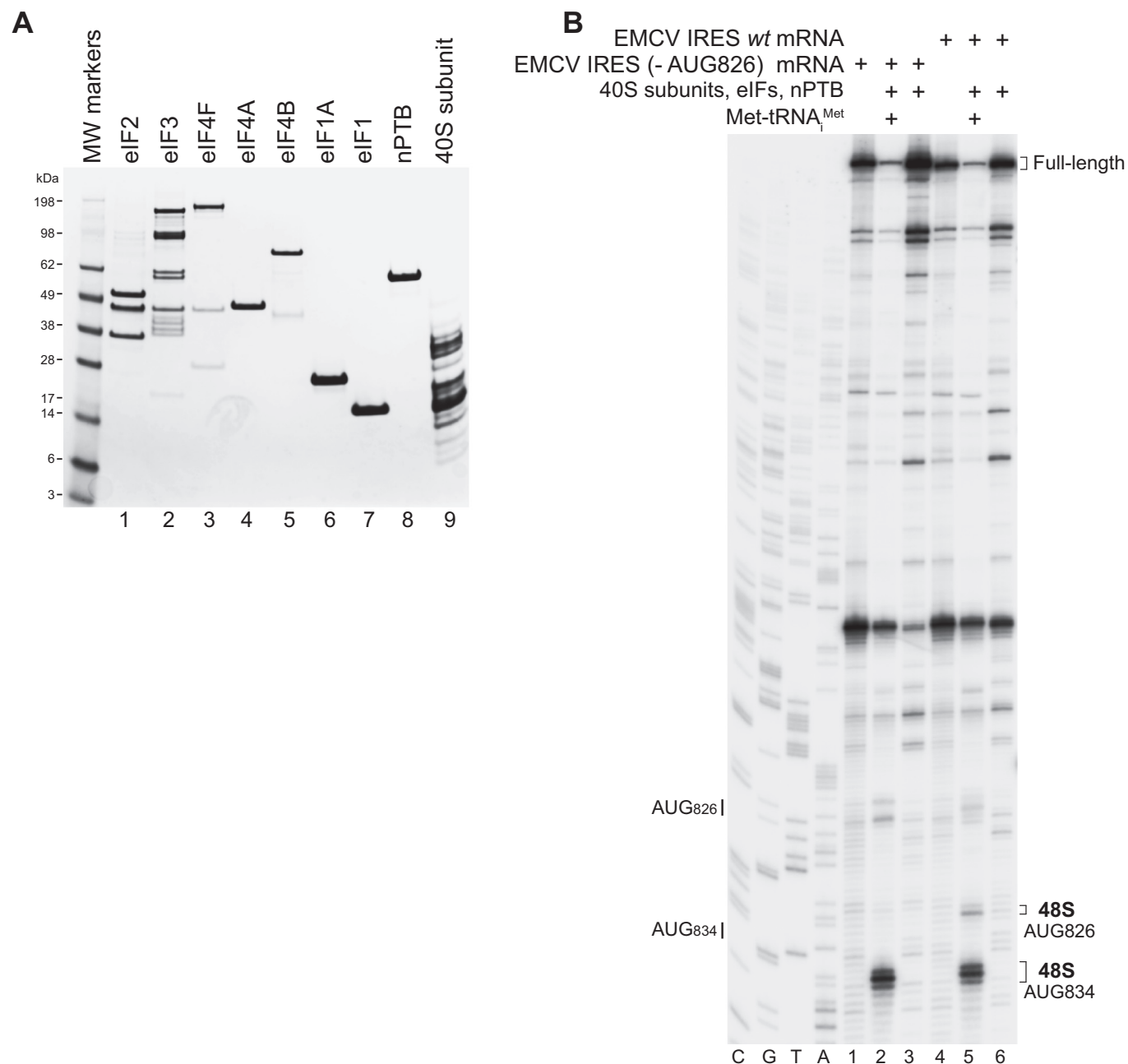


Figure EV2. 48S complex formation on the wt EMCV IRES and the IRES with mutated AUG₈₂₆.

(A) Purified initiation factors, nPTB and 40S subunits, analyzed by SDS-PAGE followed by SimplyBlue staining. (B) Toe-printing analysis of 48S complex formation on wt EMCV IRES mRNA and IRES mRNA with mutated AUG₈₂₆ in the presence of 40S subunits, initiator Met-tRNA_i^{Met}, nPTB and indicated initiation factors. Lanes C, T, A, and G depict wt EMCV sequence generated using the same primer. The positions of initiation codons are indicated on the left and of assembled 48S complexes on the right.

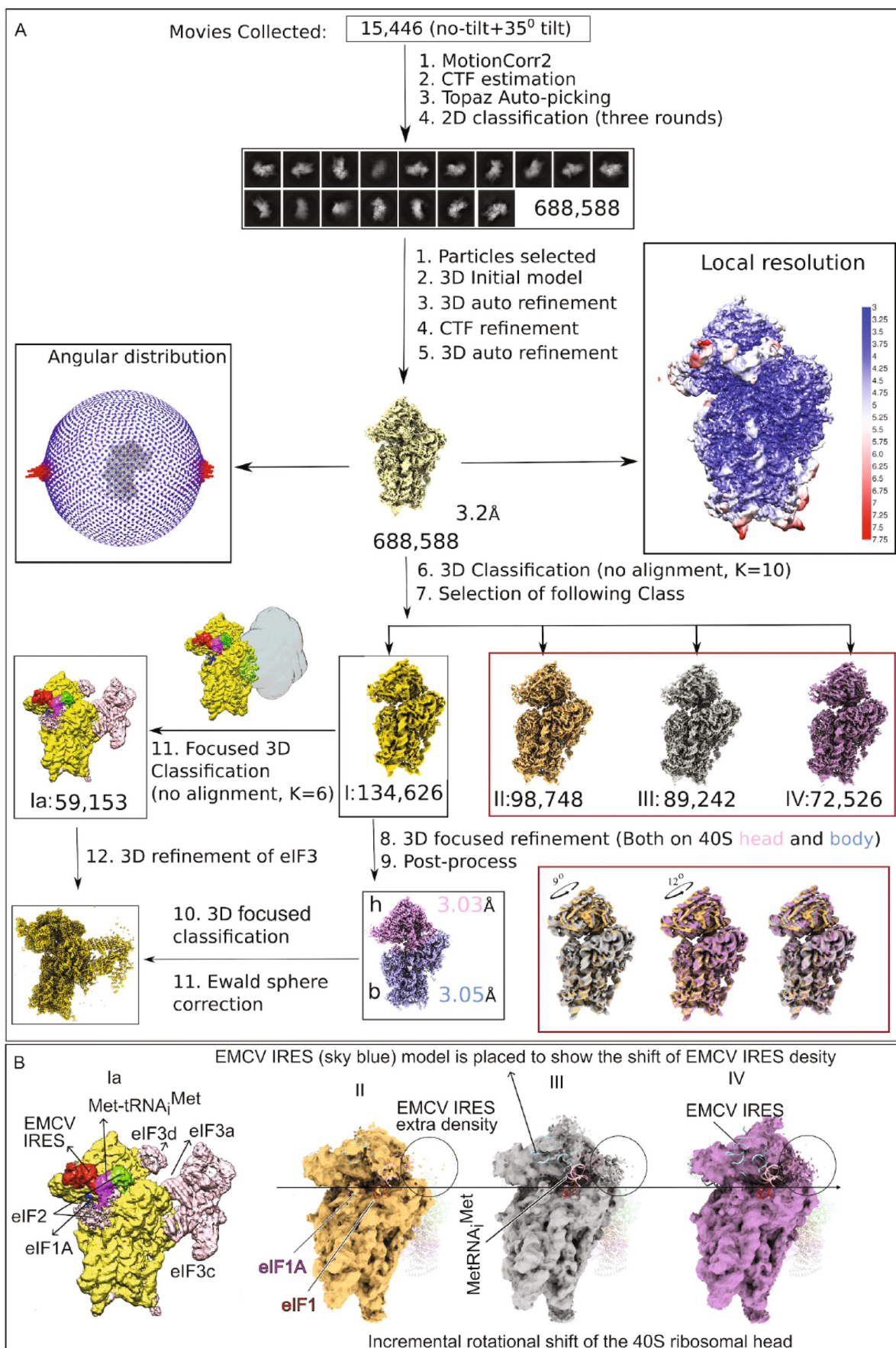


Figure EV3. Cryo-EM data processing workflow and structural characterization of EMCV IRES 48S initiation complexes.

(A) Data processing pipeline for structure determination of EMCV IRES 48S initiation complexes. (B) Class Ia (left): 48S initiation complex showing Met-tRNA^{Met}, eIF2, eIF1A, eIF3 (eIF3a/c/d subunits are indicated), and EMCV IRES density bound at the 40S subunit head. Classes II-IV: A series of reconstructions capturing progressive 40S head rotation, accompanied by repositioning of the EMCV IRES. Fragmented extra density attributed to the IRES is highlighted (circled), and a sky-blue model of the EMCV IRES is overlaid to illustrate its positional shift. Gaussian filtering reveals that this density remains connected to the IRES across all states. The horizontal arrow from left to right denotes the direction of progressive rotational movement of the 40S head, representing a continuum of structural intermediates.

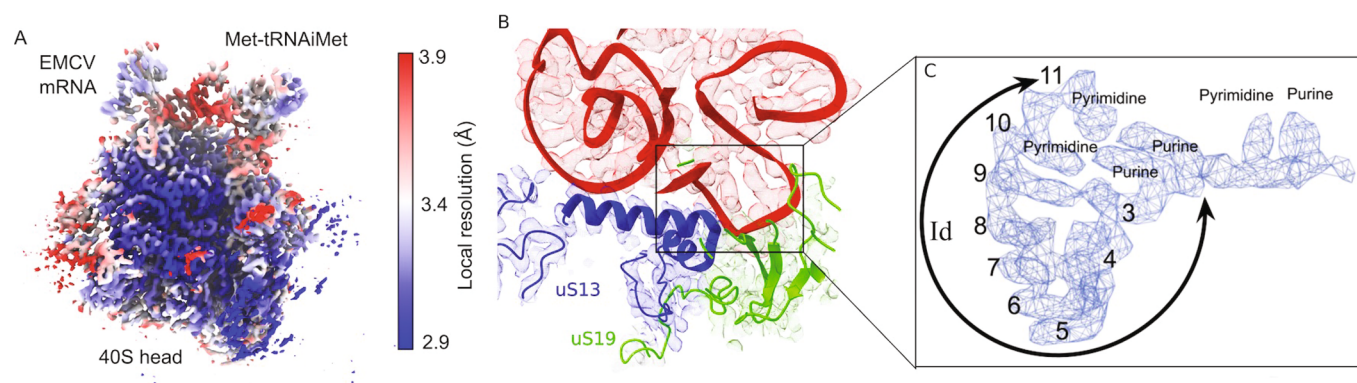


Figure EV4. Local resolution estimation and assignment of nucleotides of EMCV mRNA.

(A) The local resolution estimation of the 40S head part along with the EMCV IRES and Met-tRNA_i^{Met}. (B, C) The zoomed view of the Id subdomain of the EMCV IRES (in mesh) showing the loop formation through base pairing among RNA bases. The models fitted within densities of the EMCV IRES (red), uS19 (green), and uS13 (blue) are showing the relative position of Id on the 40S head.

