SELECTION OF PROTEINS FOR STRUCTURE DETERMINATION USING NMR

Topical category: high-throughput nuclear magnetic resonance

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A new and systematic approach to selection of structural genomics targets for structure determination using NMR spectroscopy is reported. To achieve this goal, selection based on amino acid sequence was performed (selection criteria were established towards NMR amenability), followed by protein preparation and screening using NMR spectroscopy.

Target selection criteria were applied to a starting target list of 359,085 open reading frames from 105 proteomes, of which 201 targets were selected based on amino acid sequence analysis. These targets were cloned and expressed using the JCSG pipeline, which yielded 26% of the proteins in soluble form. The soluble targets were subsequently prepared on a larger scale for NMR screening. Typically, 10 μL of protein solution were used to record 1D ^{1}H NMR spectra. Automated equipment allowed for sample loading into capillary tubes, sample exchange and spectra acquisition in a high-throughput fashion. Based on the 1D ^{1}H NMR spectra, targets were rated with 4 grades: A (folded globular proteins), B (folded globular proteins not meeting very stringent criteria applied to A-grade proteins), C (aggregated proteins) and D (non-globular proteins). This approach allowed us to identify 20 globular proteins of A- or B-grade (38% among the soluble targets). The oligomeric state of these proteins will be screened and monomeric proteins will be targets for structure determination using NMR spectroscopy.