

Selection of Proteins as Structural Genomics Targets for NMR Structure Determination

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A systematic approach to the selection of structural genomics targets for structure determination using NMR spectroscopy is reported. It includes selection based on the amino acid sequence, followed by protein preparation and screening using 1D ¹H NMR spectroscopy.

Protein properties calculated from the amino acid sequence were used to establish the initial target selection criteria. These criteria were applied to a starting target list of 430,440 open reading frames from 107 proteomes, of which 223 targets were selected based on the sequence criteria. These 223 targets were cloned and expressed using the JCSG protein production pipeline, which yielded 23% of the proteins in soluble form. The soluble targets were subsequently prepared on a larger scale for NMR screening. Typically, 10 μ L of approximately 1 mM protein solution were used to record a 1D ¹H NMR spectrum. Automated equipment allowed for sample loading into capillary tubes, sample exchange and spectra acquisition in a high-throughput fashion. This screening approach based on the 1D ¹H NMR spectra allowed us to identify 20 promising globular target proteins. Seven of these proteins were selected for further studies, and three monomeric proteins in this group are presently prioritized for structure determination using NMR spectroscopy.

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