Leaving your protein solution shaking overnight after adding Triton X-100 to the ethanolamine mix, instead of the prescribed 45 minutes, could have several potential effects on the proteins, particularly depending on the nature of the proteins you're working with, the concentration of Triton X-100, and the conditions of shaking (e.g., temperature, speed).

Here are some considerations:

1. Protein Stability:

- Potential Denaturation: Extended shaking can lead to protein denaturation, especially if the proteins are sensitive to physical stress or if the shaking induces significant temperature changes over time.
- Aggregation: Prolonged exposure to shaking might promote protein aggregation, especially in the presence of Triton X-100, which could disrupt protein-protein interactions and lead to the formation of insoluble aggregates.

2. Detergent Effects:

- Increased Solubilization: Triton X-100 is a non-ionic detergent commonly used to solubilize membrane proteins by disrupting lipid-lipid and lipid-protein interactions. Extended shaking could potentially enhance the solubilization effect, but it might also over-solubilize proteins, leading to loss of native structure and function, especially for membrane proteins.
- Micelle Formation: Extended interaction with Triton X-100 could lead to excessive micelle formation around the proteins. While this can protect hydrophobic regions of proteins, it may also interfere with subsequent purification steps by making it difficult to remove the detergent or by trapping proteins within micelles.

3. Impact on Protein Function:

- Functional Loss: For proteins that require a specific conformation to function (e.g., enzymes, receptors), extended shaking in the presence of a detergent might lead to irreversible changes in their conformation, resulting in a loss of biological activity.
- Changes in Protein-Protein Interactions: The physical and chemical environment created by prolonged shaking with Triton X-100 might alter protein-protein interactions, potentially affecting the formation of functional complexes or assemblies.

4. Complications in Subsequent Purification Steps:

- Difficulty in Removing Detergent: Removing excess Triton X-100 becomes more challenging if proteins have been excessively solubilized or if micelles trap proteins. This can complicate downstream processing steps, such as chromatography, where detergent presence can affect binding and elution profiles.
- Impact on Yield and Purity: The overall yield and purity of the target protein could be negatively affected, as prolonged treatment might lead to degradation, aggregation, or loss of the protein through nonspecific binding or precipitation.