Supporting Information

Site-specific interaction between α-synuclein and membranes probed by NMR-observed methionine oxidation rates

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Expression and purification of α-synuclein. N-terminally acetylated WT or V49M aS was expressed as described previously, using co-expression of aS and the NatB complex in E. coli.\textsuperscript{1,2} The expression was performed in protonated M9 medium supplemented with \textsuperscript{[1H,15N]} IsoGro (Sigma, St. Louis, MO). Some of the preliminary methionine oxidation experiments were conducted on perdeuterated non-acetylated WT or V49M aS, selectively protonated and \textsuperscript{13C}-labeled on methionine methyls. In this case bacteria were grown in D\textsubscript{2}O-based M9 medium including \textsuperscript{15N} ammonium chloride, and supplemented with MEM vitamins. Approximately 1 hour prior to induction 100 mg/L of [methyl-\textsuperscript{13C}] L-methionine (Cambridge Isotope Labs) was added. The resulting protein showed no significant \textsuperscript{15N} enrichment at Met backbone amide positions. Protein was purified as described previously.\textsuperscript{1} Briefly the cell lysate was subjected to heat precipitation at 85 °C; anion-exchange chromatography was performed on the supernatant; and size-exclusion chromatography was performed on the aS-containing fractions from the anion-exchange run.

Preparation of peroxidized lipids. Peroxidized lipid was produced by gamma-irradiation\textsuperscript{3} of a 1\% w/v solution of small unilamellar vesicles (SUV) prepared by sonication.\textsuperscript{4} The level of peroxidation was then further increased by adding 1 mM FeSO\textsubscript{4} for a duration of 8 h, followed by extensive dialysis for removal of the FeSO\textsubscript{4}. Initial lipid composition was 5:3:2 DOPE/DOPS/DOPC (coagulation reagent I from Avanti Polar Lipids). After gamma-irradiation the SUVs were dialyzed against water and lyophilized. The resulting powder was later mixed in 1:1 ratio with pure DOPC (Avanti Polar Lipids) in chloroform and dried. The resulting lipid had the composition of approximately 15\% DOPS, 25\% DOPE, and 60\% DOPC. This mixture was used to make a 10\% stock solution of SUVs, which was subsequently used to set up methionine oxidation reactions. The concentration of lipid peroxides in the resulting SUVs was determined using the Lipid Hydroperoxide Assay Kit from Cayman Chemical. The fraction of lipid peroxides
was found to be ca. 10% of the total amount of oleyl fatty acid chains for the lipid preparation used for WT aS, and 8.8% for the lipid preparation used for the V49M mutant.

**Oxidation reaction conditions.** Oxidation reactions were set up with 100 µM acetylated WT or V49M aS. Buffer conditions were: 20 mM Na phosphate pH 6, 100 mM NaCl. Lipid concentrations used for the three V49M reactions were 5% w/v (L:P ~ 630), 2.4% w/v (L:P ~ 300), and 1.2% w/v (L:P ~ 150). Lipid composition was approximately 15% DOPS, 25% DOPE, and 60% DOPC (as mentioned above), with the concentration of oxidized lipids of ~8.8% for V49M and ~10.0% for WT aS. Reaction mixtures were kept at room temperature in closed Falcon tubes, and 300 µL aliquots were taken at defined time points for analysis.
Fitting of Met oxidation data for WT aS

Met oxidation in aS is described by Scheme S1.

![Scheme S1](image)

**Scheme S1.** Pathways leading to formation of all observed methionine-oxidized species of WT aS.

Here NN, ON, NO, and OO represent the populations of aS molecules in different states of oxidation: NN – not oxidized, ON – only M1 is oxidized, NO – only M5 is oxidized, and OO – both M1 and M5 are oxidized. The parameters $k_{X-Y}$ denote oxidation rates from state X to state Y.

The oxidation reaction was carried out at 630:1 lipid to protein molar ratio. With the fraction of peroxidized lipids measured to be ca. 10%, and considering that every lipid molecule contains two unsaturated oleyl chains, this corresponds to a 130:1 molar ratio of LOOH to protein. Even though each aS molecule has two Met residues participating in the reaction, the decrease in the total amount of LOOH associated with full oxidation of both Met1 and Met5 is only 1.5%. Thus, under these conditions aS oxidation can be treated as a first-order reaction:

\[
\begin{align*}
\frac{dNN}{dt} &= -(k_{NN-ON} + k_{NN-NO})NN \\
\frac{dON}{dt} &= k_{NN-ON}NN - k_{ON-OO}ON \\
\frac{dNO}{dt} &= k_{NN-NO}NN - k_{NO-OO}NO \\
\frac{dOO}{dt} &= k_{ON-OO}ON + k_{NO-OO}NO
\end{align*}
\]  

(S1)
This system of differential equations can be easily integrated numerically, given the values of $k_{X,Y}$ and setting the initial conditions $NN = 1$, $ON = NO = OO = 0$ at $t=0$. Fitting the experimental data to predicted curves then involves minimization of the following error function:

$$E = \sum_{X=NN,ON,NO} \sum_{i=1}^{n} \frac{(X_{\text{exp}}(t_i) - X_{\text{pred}}(t_i))^2}{\Delta^2}$$

where $\Delta$ is experimental error, estimated to be 0.01 on the basis of a 100:1 S/N in the spectrum of the fully reduced protein. The inner sum is performed over all experimental time points (in our case $n=8$). Notice that the outer summation doesn’t include the population of the state $OO$. This accounts for the fact that, consistently with Eq S1, the total population must always be equal to unity ($NN+ON+NO+OO=1$), meaning that the populations of one of the four species cannot be considered independent.

Below, three different models, involving different numbers of adjustable parameters, are evaluated for fitting the experimental data. The uncertainties in the solutions found from the data fitting procedures are determined using a Monte-Carlo approach, where normally distributed random numbers with a standard deviation matching the experimental uncertainty ($\Delta=0.01$) were added to all the experimental intensities. For each model the procedure was executed 200 times; the results of each run were stored, and the stored values were subsequently used to calculate the mean values and standard deviations of the fitting parameters.

**Model 1.** The oxidation rates of M1 and M5 are different, but are not dependent on the oxidation state of the other methionine. In this case we set $k_{NN-ON} = k_{NO-OO} = k_{M1}$ and $k_{NN-NO} = k_{ON-OO} = k_{M5}$.

Results: $k_{M1} = 0.235 \pm 0.003 \text{ h}^{-1}$, $k_{M5} = 0.148 \pm 0.002 \text{ h}^{-1}$, $E = 545$. 

**S5**
The quality of fitting, expressed by the error function $E$, is very poor, as also can be seen from visual inspection of the corresponding best-fitted curves (data not shown). This result was expected as Met oxidation decreases lipid affinity of aS (Figure 2, main text), which should translate into slowdown of the oxidation rate of the second Met residue once the first one has become oxidized.

**Model 2.** All oxidation rates $k_{X-Y}$ are considered independent parameters.

Results: $k_{NN-ON} = 0.259 \pm 0.005 \text{ h}^{-1}$, $k_{NN-NO} = 0.174 \pm 0.005 \text{ h}^{-1}$, $k_{ON-OO} = 0.110 \pm 0.004 \text{ h}^{-1}$, $k_{NO-OO} = 0.124 \pm 0.006 \text{ h}^{-1}$, $E = 28.1$.

This model provides the best fitting possible under the assumption that Eq S1 gives a perfect description of the process. The initial oxidation rate of M1 is substantially faster than that of M5 (by about 50%). Considering the pairs of rates corresponding to oxidation of M1 or M5, we find that their ratios fall quite close to each other: $k_{NO-OO} / k_{NN-ON} = 0.48$ and $k_{ON-OO} / k_{NN-NO} = 0.64$. We call these ratios slowdown factors and introduce the next model.

**Model 3.** The initial oxidation rates of M1 and M5 are different, but their slowdown factors are the same. In this case the shared slowdown factor $\mu = k_{NO-OO} / k_{NN-ON} = k_{ON-OO} / k_{NN-NO}$ is used as an independent fitting parameter.

Results: $k_{NN-ON} = 0.251 \pm 0.005 \text{ h}^{-1}$, $k_{NN-NO} = 0.184 \pm 0.005 \text{ h}^{-1}$, $\mu = 0.55 \pm 0.02$, $E = 34.3$. 
Comparison of the models

Table S1. Quality of fit to experimental data obtained for the three proposed models describing the time course of methionine oxidation for WT aS.

| Model number | Number of parameters, \( m \) | Number of degrees of freedom \( N \) | \( E \) | \( E / N \) |
|--------------|-------------------------------|-------------------------------------|-------|---------|
| 1            | 2                             | 22                                  | 545   | 24.7    |
| 2            | 4                             | 20                                  | 28.1  | 1.4     |
| 3            | 3                             | 21                                  | 34.3  | 1.6     |

It is clear that model 1 is unacceptable. It is more interesting to see whether the difference between models 2 and 3 is statistically significant. The corresponding F-test value is \( F = (34.3 – 28.1) / (28.1/20) = 4.43 \), and the \( P \) value is \( P(4.43, 1, 20) = 0.05 \). This \( P \) value falls above the typical 0.01 cutoff for statistical significance. Although there is no reason to believe that model 3 should give a better description of the reaction, our analysis shows that the number of fitting parameters can be reduced by one (by introducing the slowdown factor \( \mu \)) without substantially worsening the quality of fit.

Fitting of Met oxidation data for V49M mutant of aS

Addition of M49 considerably complicates the description of the oxidation reaction by increasing the number of different oxidized species to 8. The potential number of distinct oxidation rates \( k_{X,Y} \) then increases to 12. This number of fitting parameters is too high to lead to a unique solution for the fitting problem. Therefore, we decreased the number of fitting parameters relying on certain considerations rooted in experimental observations, and expanded the application of “slowdown factors” similar to \( \mu \), introduced above. The main assumption we make is that M1 and M5 behave
identically in the degree of slowdown that they can induce or experience. For example, the rates of M49 oxidation from state NON to NOO, and from state ONN to ONO are presumed to be the same, and equal to $\alpha$ times the rate of oxidation from NNN to NNO.

Again treating aS oxidation as a first-order reaction the following system of equations gives time evolution of all species:

$$
\begin{align*}
\frac{d\text{NNN}}{dt} &= -(k_{M1} + k_{M5} + k_{M49})\text{NNN} \\
\frac{d\text{NNO}}{dt} &= -\gamma \cdot (k_{M1} + k_{M5})\text{NNO} + k_{M49}\text{NNN} \\
\frac{d\text{ONN}}{dt} &= k_{M1}\text{NNN} - \mu \cdot k_{M5}\text{ONN} - \alpha \cdot k_{M49}\text{ONN} \\
\frac{d\text{ONO}}{dt} &= \gamma \cdot k_{M1}\text{NNO} - \gamma_2 \cdot \mu \cdot k_{M5}\text{ONO} + \alpha \cdot k_{M49}\text{ONN} \\
\frac{d\text{NON}}{dt} &= k_{M5}\text{NNN} - \mu \cdot k_{M1}\text{NON} - \alpha \cdot k_{M49}\text{NON} \\
\frac{d\text{NOO}}{dt} &= \gamma \cdot k_{M5}\text{NNO} - \gamma_2 \cdot \mu \cdot k_{M1}\text{NOO} + \alpha \cdot k_{M49}\text{NON} \\
\frac{d\text{OON}}{dt} &= \mu \cdot k_{M5}\text{ONN} + \mu \cdot k_{M1}\text{NON} - \beta \cdot k_{M49}\text{OON} \\
\frac{d\text{OOO}}{dt} &= \gamma_2 \cdot \mu \cdot k_{M5}\text{ONO} + \gamma_2 \cdot \mu \cdot k_{M1}\text{NOO} + \beta \cdot k_{M49}\text{OON}
\end{align*}
$$

Here $k_{M1}$, $k_{M5}$, and $k_{M49}$ are the oxidation rates of M1, M5, and M49 respectively from state NNN, while all the other $k_{X\cdot Y}$ rates are expressed as one of these three rates multiplied by relevant slowdown factors. The slowdown factors of Equation 3 are defined as follows:

- $\alpha$ – slowdown in the oxidation rate of M49 when either M1 or M5 is oxidized (but not both)
- $\beta$ – slowdown in the oxidation rate of M49 when both M1 and M5 are oxidized
- $\mu$ – defined as in model 3 for WT, i.e. the slowdown of M1 (M5) caused by oxidation of M5 (M1) when M49 is not oxidized
- $\gamma$ – slowdown of the oxidation rates of M1 and M5 caused by oxidation of M49, when M1 and M5 are not initially oxidized
\( \gamma_2 \) – additional slowdown of the oxidation rate of M1 (M5) caused by oxidation of M49, when M5 (M1) is already oxidized. This factor only shows up in the combination \( \mu \cdot \gamma_2 \) to enforce the idea that the total slowdown is at least as strong as \( \mu \), and possibly stronger if \( \gamma_2 < 1 \).

The importance of varying all of the parameters \( \beta, \gamma, \) and \( \gamma_2 \) independently (or at all) is not known a priori. The importance of the slowdown factor \( \mu \) was established from fitting WT oxidation data. Similarly, the factor \( \alpha \) is important, because it describes the rapid slowdown in M49 oxidation with time observed in the experimental data (Figure 3B, main text).

An additional complication compared to the case of WT aS arises from the fact that we cannot directly observe the populations of all 8 distinct oxidation states of V49M by NMR. Indeed M49 is located so far from M1 and M5 in sequence that its oxidation does not affect the peak positions in the vicinity of M1 and M5. Vice versa, oxidation of M1 or M5 does not affect the resonance positions of amides in the vicinity of M49. Consequently we can only directly observe “cumulative populations” \( \text{NN} = \text{NNN} + \text{NNO}, \quad \text{ON} = \text{ONN} + \text{ONO}, \quad \text{NO} = \text{NON} + \text{NOO}, \quad \text{OO} = \text{OON} + \text{OOO}, \quad \text{XN} = \text{NNN} + \text{ONN} + \text{NON} + \text{OON}, \quad \text{XO} = \text{NNO} + \text{ONO} + \text{NOO} + \text{OOO}. \) Since \( \text{NN} + \text{ON} + \text{NO} + \text{OO} = 1 \) and \( \text{XN} + \text{XO} = 1 \) by definition, we treat only four of the experimental curves as independent: NN, ON, NO, and XN. Thus the definition of the error function used for data fitting is modified slightly compared to Eq S2:

\[
E = \sum_{X=\text{NN}, \text{ON}, \text{NO}, \text{XN}} \frac{1}{\Delta^2} \sum_{i=1}^{n} \left( X_{\text{exp}}(t_i) - X_{\text{pred}}(t_i) \right)^2
\]

Again, we fit the experimental data to several different models, two of which are described in more detail below. The mean and standard deviation of fitting parameters were calculated using the same Monte-Carlo approach described above for fitting of the WT data.
**Model 1.** Initial oxidation rates of M1, M5 and M49 are different; M1 and M5 affect each other and M49, but M49 doesn’t affect M1 and M5. Independent fitting parameters are \( k_{M1}, k_{M5}, \mu, k_{M49}, \alpha \). We set \( \beta = \alpha, \gamma = \gamma_2 = 1 \).

The reason for considering this model as potentially adequate is the knowledge that the most N-terminal fragment of aS is pivotal in promoting lipid binding.\(^1,5\) Thus, one would expect oxidation of M1 and M5 to be much more important than oxidation of M49. The assumption \( \beta = \alpha \) implies that oxidation of either M1 or M5 has as strong an effect on M49 oxidation as does oxidation of both M1 and M5 simultaneously. While this appears to contradict our observations of the effect of lipid titration on different oxidized species (Fig. 2, main text), we found that making \( \beta \) an independent parameter has a negligible effect on the quality of fit.

The results of fitting the time dependence of the observed intensities for the oxidation reactions with different lipid to protein (L:P) ratios are presented in Table S2.

**Table S2.** Values of fitting parameters obtained by applying "Model 1" to the three data sets measured for methionine oxidation of N-terminally acetylated V49M mutant of aS.

| L:P  | \( k_{M1} \)     | \( k_{M5} \)     | \( \mu \)     | \( k_{M49} \)  | \( \alpha \)  | E    |
|------|------------------|------------------|--------------|----------------|--------------|------|
| 150:1| 0.068±0.001      | 0.047±0.001      | 0.37±0.01    | 0.071±0.002    | 0.32±0.02    | 54.1 |
| 300:1| 0.121±0.002      | 0.084±0.002      | 0.45±0.01    | 0.134±0.005    | 0.34±0.02    | 67.0 |
| 630:1| 0.179±0.003      | 0.136±0.003      | 0.58±0.02    | 0.209±0.006    | 0.41±0.02    | 103.3|

**Model 2.** Initial oxidation rates of M1, M5 and M49 are different; M1 and M5 affect each other as well as M49, and oxidation of M49 has an effect on M1 and M5. Independent fitting parameters are \( k_{M1}, k_{M5}, \mu, k_{M49}, \alpha, \gamma \). We set \( \beta = \alpha, \gamma_2 = 1 \).
While the possibility that M49 oxidation can have an effect on M1 and M5 was not anticipated, only by introducing an adjustable parameter $\gamma < 1$ can an adequate fit be obtained at high lipid:aS stoichiometry.

The results of fitting the time dependence of the observed intensities for the oxidation reactions with model 3 for the different lipid to protein (L:P) ratios are presented in Table S4.

**Comparison of the models**

**Table S3.** Quality of fit to three sets of experimental data obtained for the two proposed models describing the time course of methionine oxidation for V49M mutant of aS.

| Model number | Number of parameters, $m$ | L:P   | Number of degrees of freedom $N^*$ | $E$  | $E / N$ |
|--------------|--------------------------|-------|-----------------------------------|------|---------|
| 1            | 5                        | 150:1 | 27                                | 54.1 | 2.0     |
|              |                          | 300:1 | 23                                | 67.0 | 2.9     |
|              |                          | 630:1 | 23                                | 103.3| 4.5     |
| 2            | 6                        | 150:1 | 26                                | 50.1 | 1.9     |
|              |                          | 300:1 | 22                                | 54.3 | 2.5     |
|              |                          | 630:1 | 22                                | 46.8 | 2.1     |

*Note that 8 time points were taken for the 150:1 dataset, and 7 time points were taken for the other two datasets.

The introduction of the effect of M49 oxidation on M1 and M5 oxidation rates is most significant for the fit of the data measured at high lipid:aS ratio (630:1), when competition for limited space on the surface of the SUVs is minimal. Again, an F-test is used to evaluate whether the decrease in the error function is statistically significant. The F-test value $F = (103.3 - 46.8) / (46.8/22) = 26.9$,
with the corresponding $P$ value $P(26.9, 1, 22) = 3.4 \times 10^{-5}$ demonstrating that the improvement is indeed statistically significant, and that model 2 must be used for an adequate description of the V49M aS oxidation reaction.

**Table S4.** Values of fitting parameters obtained by applying "Model 2" to the three data sets measured for methionine oxidation of the N-terminally acetylated V49M mutant of aS.

| L:P$^a$ | $k_{M1}$ | $k_{M5}$ | $\mu$ | $\gamma$ | $k_{M49}$ | $\alpha$ | $E$  |
|--------|----------|----------|-------|---------|----------|---------|-----|
| 150:1  | 0.071±0.002 | 0.048±0.002 | 0.36±0.01 | 0.84±0.05 | 0.071±0.002 | 0.32±0.01 | 50.1 |
| 300:1  | 0.131±0.004 | 0.091±0.003 | 0.42±0.02 | 0.73±0.06 | 0.135±0.004 | 0.35±0.02 | 54.3 |
| 630:1  | 0.209±0.007 | 0.159±0.006 | 0.48±0.02 | 0.55±0.06 | 0.217±0.006 | 0.41±0.02 | 46.8 |

$^a$ Lipids are 15% DOPS, 25% DOPE, and 60% DOPC, with 8.8±0.5% oleyl peroxidation. For other sample conditions, see caption to Figure 1, main text.
Figure S1. Effect of progressive Met oxidation on interaction of V49M aS (non-acetylated) with lipids. Plotted are the ratios of aS $^{15}$N-HSQC peak heights in the presence ($I$) and absence ($I_0$) of lipids at three time points during an oxidation reaction: 2 hours (blue), 16 hours (green), and 36 hours (red). The progressive decrease in intensity of the 3-12 and 44-54 regions results from the shift of peak intensities into new positions. The progressive increase in the intensities of the other N-terminal peaks results from decreased lipid binding of the oxidized species of aS. Sample conditions: 150 μM perdeuterated V49M, 0.18% w/v DOPG SUV (lipid:protein = 15), 0.5 M NaCl, 20 mM Na phosphate, pH 6, 15 °C. The methionine oxidation reaction was monitored in real time by recording $^1$H-$^{15}$N TROSY-HSQC spectra on perdeuterated aS at very high resolution every 2 hours on an 800 MHz spectrometer, with acquisition times of 350 ms and 164 ms in the $^1$H and $^{15}$N dimensions, respectively.
Figure S2. $^1$H-$^{13}$C HSQC spectrum collected on a sample of perdeuterated, non-acetylated, Met $^{13}$C$_\varepsilon$-labeled WT aS. The sample was incubated with SUVs containing peroxidized lipids, until completion of Met oxidation reaction was reached as judged by NMR. The residual lipids were removed by methanol precipitation of the protein. The inset shows the magnified region of the spectrum containing methionine sulfoxide peaks. Sample conditions were: 200 μM aS, 20 mM Na phosphate pH 6, 15 °C. The spectrum was acquired on a cryoprobe-equipped 500 MHz spectrometer, using acquisition times of 300 ms in the $^1$H dimension and 28 ms in the indirect $^{13}$C dimension. The methyl correlations for M116 and M127 are not affected by incubation with peroxidized lipids. Non-equivalence of the Met-R-O and Met-S-O stereoisomers of Met1 and Met5 result in a total of four $^1$H-$^{13}$C correlations (inset), two of which overlap.
Figure S3. Selective action of MsrA on Met-S-sulfoxide observed by NMR. TROSY-HSQC spectra were collected on a sample of perdeuterated, $^{13}$Cε-methyl Met labeled V49M aS, for which Met1, Met5, and Met49 had been fully oxidized using lipid peroxides. The protein was separated from lipids by several cycles of methanol precipitation prior to the start of the experiments. Peaks for F4 and V48 in the initial state of the sample are shown in the top panels: the peaks are split into doublets by oxidized M5 and M49 respectively. After the addition of MsrA, the peaks corresponding to Met5-S-O and Met49-S-O lose intensity over time. In case of V48 the intensity shifts from the S peak to the peak corresponding to reduced M49. Analogously, for F4, the intensity of the peak corresponding to reduced M5 grows in. However this resonance, labeled ON, appears as a doublet, with its components corresponding to the Met1-R-O and Met1-S-O oxidations states, with Met1 in non-acetylated aS being a poor substrate for MsrA. This is confirmed by the L8 resonance, where only a single new peak corresponding to ON appears in the course of the reaction, with the effect of Met1-R-O and Met1-S-O being unresolvable at the larger distance from the N-terminus. The experimental conditions were: 100 μM V49M aS, 0.2 μM MsrA, 5 mM dithiothreitol, 1 mM diethylene triamine pentaacetic acid (DTPA), 20 mM Na phosphate pH 6, 288 K. TROSY-HSQC spectra were taken every hour on a 900 MHz spectrometer with acquisition times of 350 ms and 220 ms in the direct and indirect dimensions respectively.
Figure S4. M1 serves as a good substrate for MsrA only when aS is N-terminally acetylated. Fragments of TROSY-HSQC spectra showing the peaks of residue L8 recorded on (A) non-acetylated and (B) acetylated aS. In both cases, results are shown for the oxidized V49M mutant after 15 hours of enzymatic reaction with MsrA. Before the initiation of the reaction both proteins were in the OOO state. Panel (A) was obtained from the same spectrum as the bottom panels of Figure S3. The fact that only the peak ON appears in this spectrum indicates that MsrA only significantly reacted with M5 on this time scale. On the other hand, in panel (B) all the possible (partially) reduced peaks grow in, indicating MsrA acts on both M1 and M5. However, even in this case the reaction rate is clearly higher for M5. The reaction conditions were identical for both samples: 100 μM aS, 0.2 μM MsrA, 5 mM dithiothreitol, 1 mM diethylene triamine pentaacetic acid (DTPA), 20 mM Na phosphate pH 6, 288 K. The spectrum of panel (A) was recorded on perdeuterated (^{13}C^{ε}-Met labeled) ^{15}N-enriched V49M aS on a 900 MHz spectrometer at very high resolution (see Figure S3). The spectrum of panel (B) was recorded on protonated, ^{15}N-enriched V49M aS on a 750 MHz spectrometer, using acquisition times of 80 ms and 170 ms in the ^{1}H and ^{15}N dimensions respectively.
Figure S5. Circular dichroism data for the interaction of N-terminally acetylated WT aS with SUVs containing the oxidized lipids used in methionine oxidation reactions. (A) Spectra are shown for lipid to protein molar ratios of 0 (black), 150:1 (blue), 300:1 (green), and 630:1 (red). (B) Graph showing the corresponding change in CD signature at 222 nm, reflecting the amount of α-helical structure, as a function of increasing lipid:protein ratio. Sample conditions: 20 mM Na phosphate pH 6, 150 mM NaCl, 5 μM N-terminally acetylated aS. The lipids consisted of 15% DOPS, 25% DOPE, 60% DOPC, and were ~10% oxidized.

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