Electronic Supplementary Material (ESI) for MedChemComm.


Supporting Information for

# Set-up and screening of a fragment library targeting 14-3-3 protein interface

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**Physico-chemical Filtering**

Physico-chemical properties have been calculated by ChemAxon JChem for Excel (version 16.10.1700.1231).

**Fragments' quality control and purification**

The initial purity of each fragment was analysed by uHPLC-MS integrated systems. For each sample, 100 mg were weighted and dissolved in a mixture of Acetonitrile and Methanol in ratio 1:1. The non-soluble fragments were excluded from the collection. uHPLC-MS checks were performed using uHPLC Agilent Technologies 1290 Infinity coupled with Agilent Technologies 6120 Quadrupole LC/MS, Column: ACQUITY UPLC®BEH C18 1.7 µm, mobile phase: mixture of Acetonitrile + 0.1% Formic Acid and Water + 0.1% Formic Acid. When purity was below the 85% threshold, preparative HPLC was performed using UPLC Agilent Technologies 1260 Infinity coupled with Agilent Technologies 6120 Quadrupole LC/MS, Column: XBridge EH C18 5 µm, 19 mm x 150 mm, mobile phase: mixture of Acetonitrile + 0.1% Formic Acid and Water + 0.1% Formic Acid.

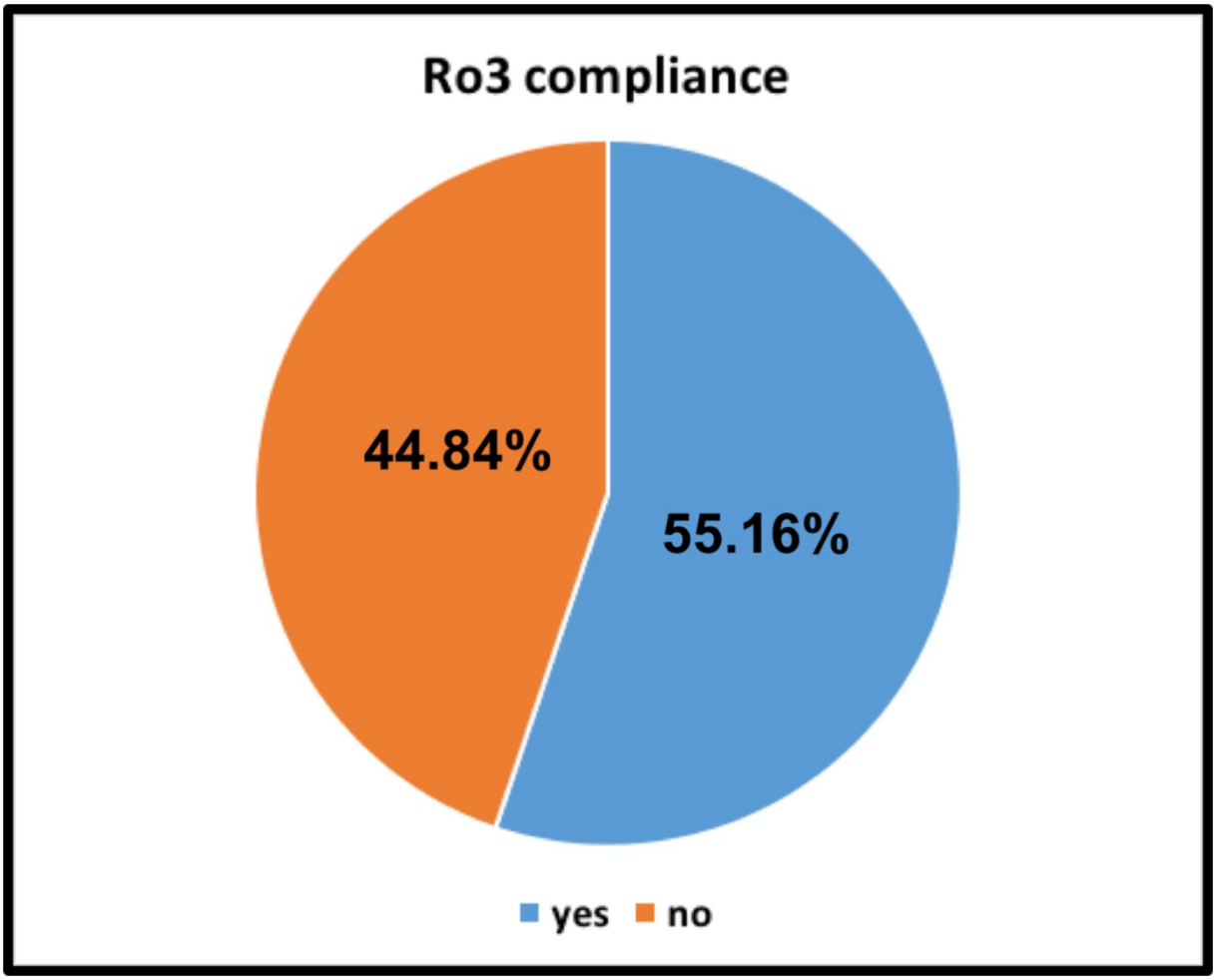


**Chart S1.** Ro3 compliance of the fragment collection.

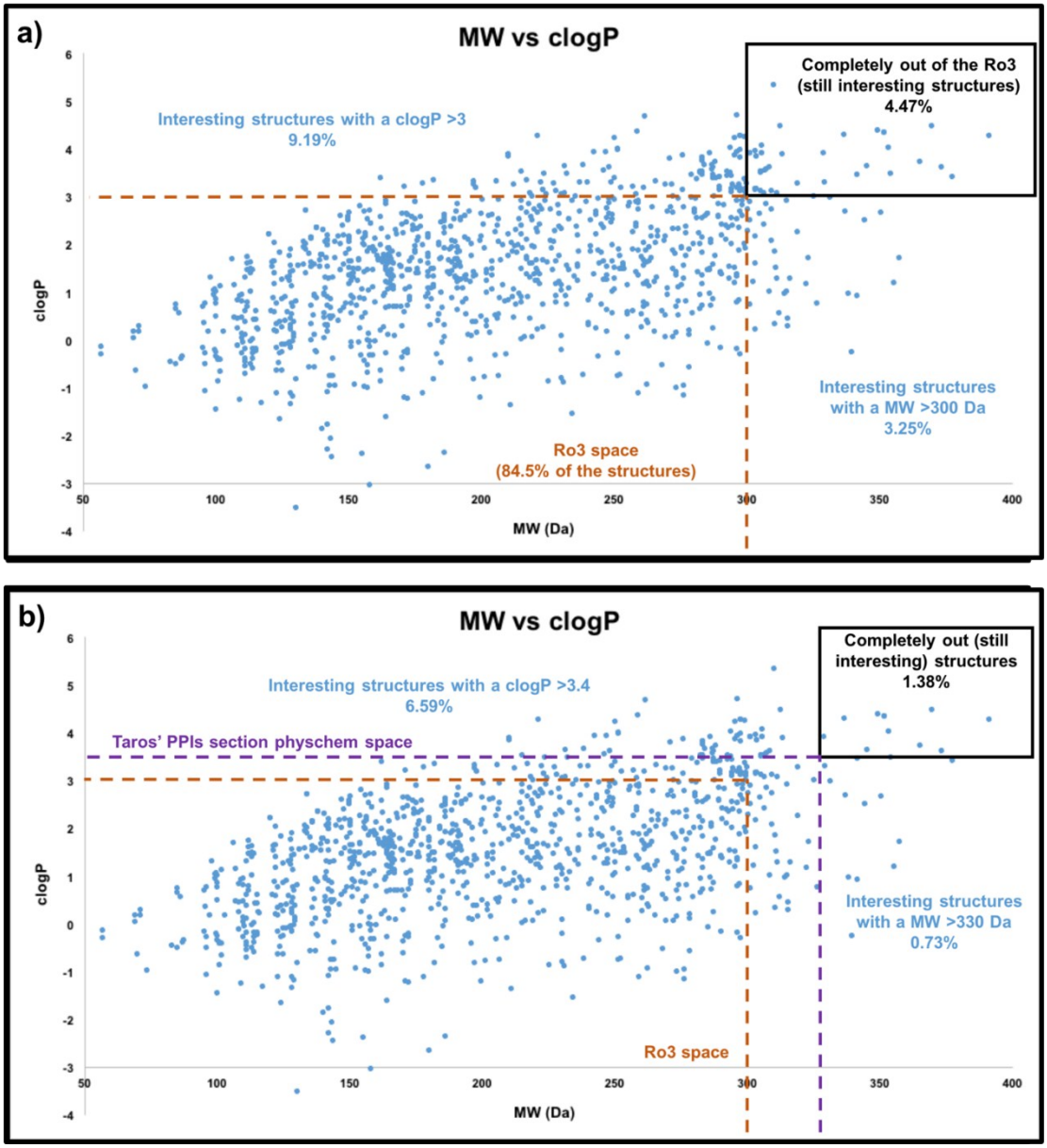


**Figure S1.** Fragments' MW and clogP distribution. Correlation between MW and clogP considering the classical Rule of Three parameters (A) or the revised limits (MW 330 Da and clogP 3.4) used for setting up the collection (B). Orange dashed line delimitates the Rule of Three (Ro3) space that is included into the wider parameters selected for this particular subset of the Taros' fragment library (purple dashed lines, (B)) while the black box on the top right includes the entries with both MW and clogP out of the Ro3 space (A) or out of the Taros' PPIs physchem space (B). (A) 12% of the structures have at least one parameter out of the rule and only 4% of the entries have both MW and clogP outside of the rule but present promising structures. (B) The percentage of structures with MW or clogP parameters over the settings decrease to 7.3% (compared to (A)) while 1.4% present both MW and clogP over the set limits but nonetheless have attractive structures.

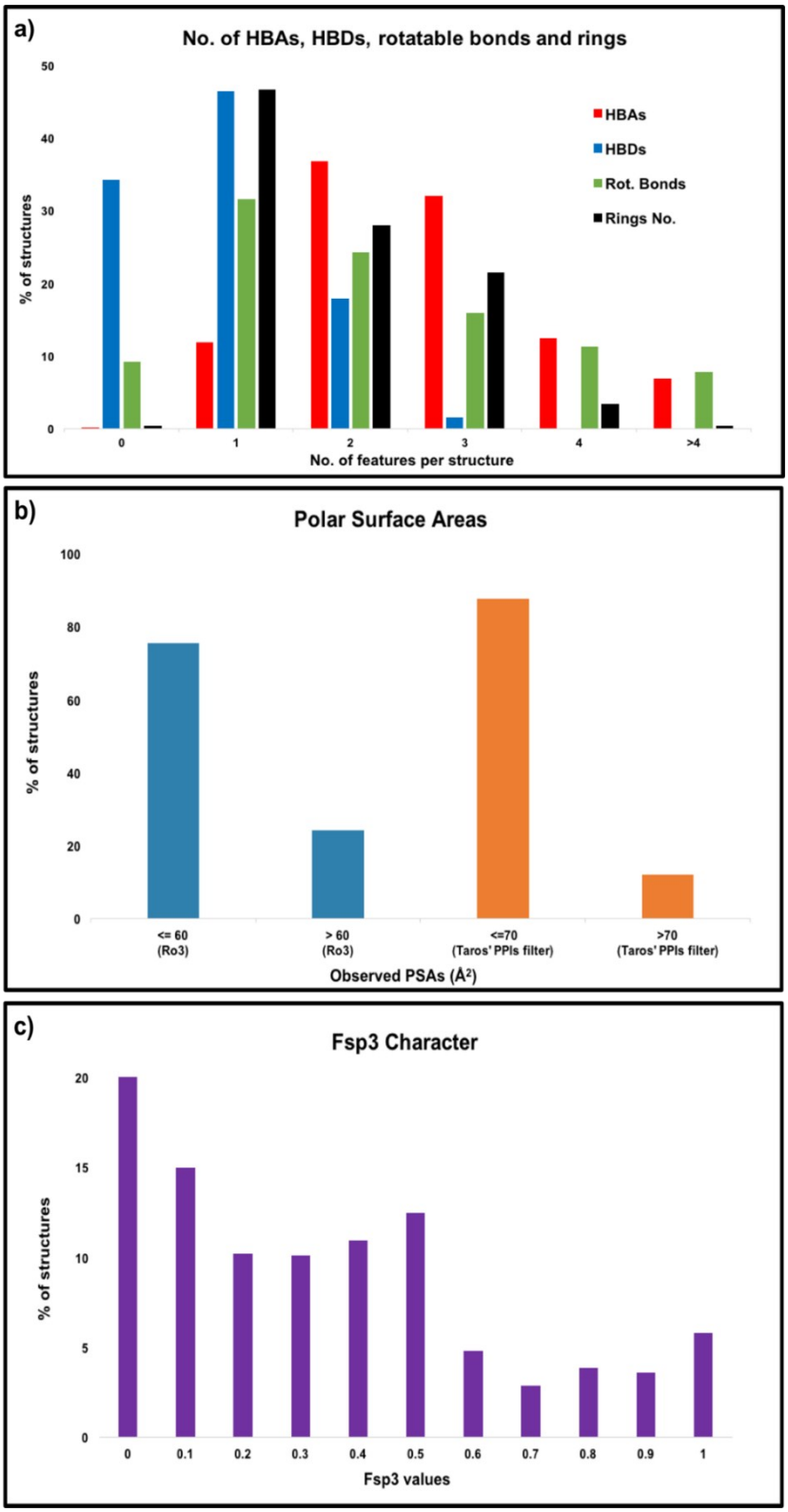


**Figure S2.** (A) Number of H-bond acceptors (HBAs), donors (HBDs), rotatable bonds (Rot. Bonds) and rings. The totality of the molecules presents no more than 3 HBDs while 80.7% has no more than 3 HBAs. Number of entries with no more than 3 rotatable bonds covers a similar percentage (80.9%). 96.4% of the structures contains no more than 3 rings. (B) Distribution of Polar Surface Areas, according to the Ro3 (cyan) and to the Taros' PPIs section parameters (orange). 75.7% has a PSA value below or equal the 60 Å$^2$ threshold. According to our parameters (PSA≤70 Å$^2$) 87.9% of the molecules fit well the requirements. (C) Saturation character (Fsp3) distribution. More than half of the structures (55.8%) present a completely flat core while 28.2% have an intermediate saturation character and 16.1% of them show a fully three-dimensional character.

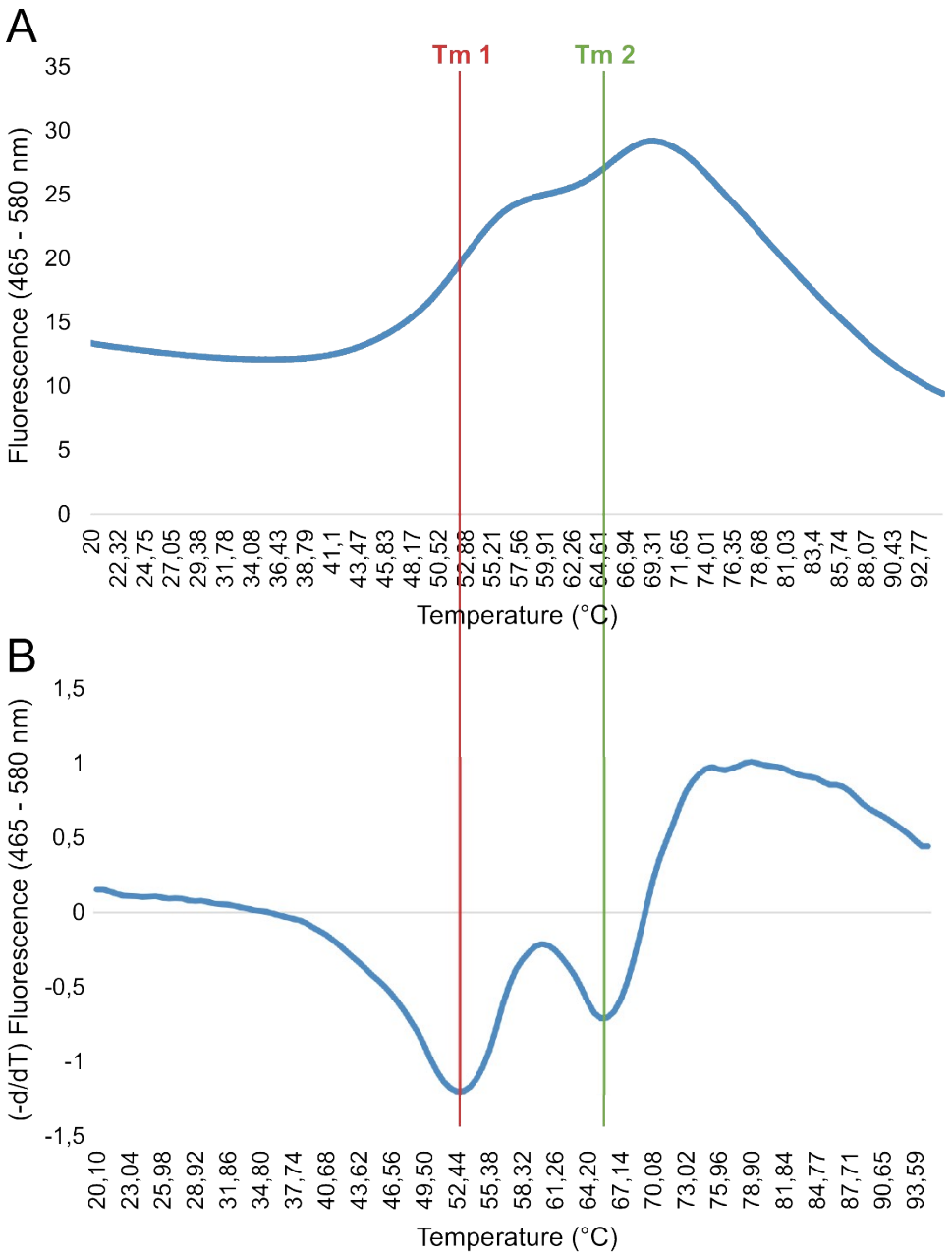


**Figure S3.** DSF analysis of melting temperatures of 14-3-3σ in the presence of compounds. The melting curve of 14-3-3σ 7 µM in the presence of 4% DMSO is presented in A as the variation of fluorescence (*y* axis) as a function of the temperature (*x* axis). Two different melting temperatures, identified as Tm 1 (in red) and Tm 2 (in green) are observed in the melting curve of the protein. The first derivative of the melting curve is presented in B. The *x* axis represents the temperature, same scale as in A.

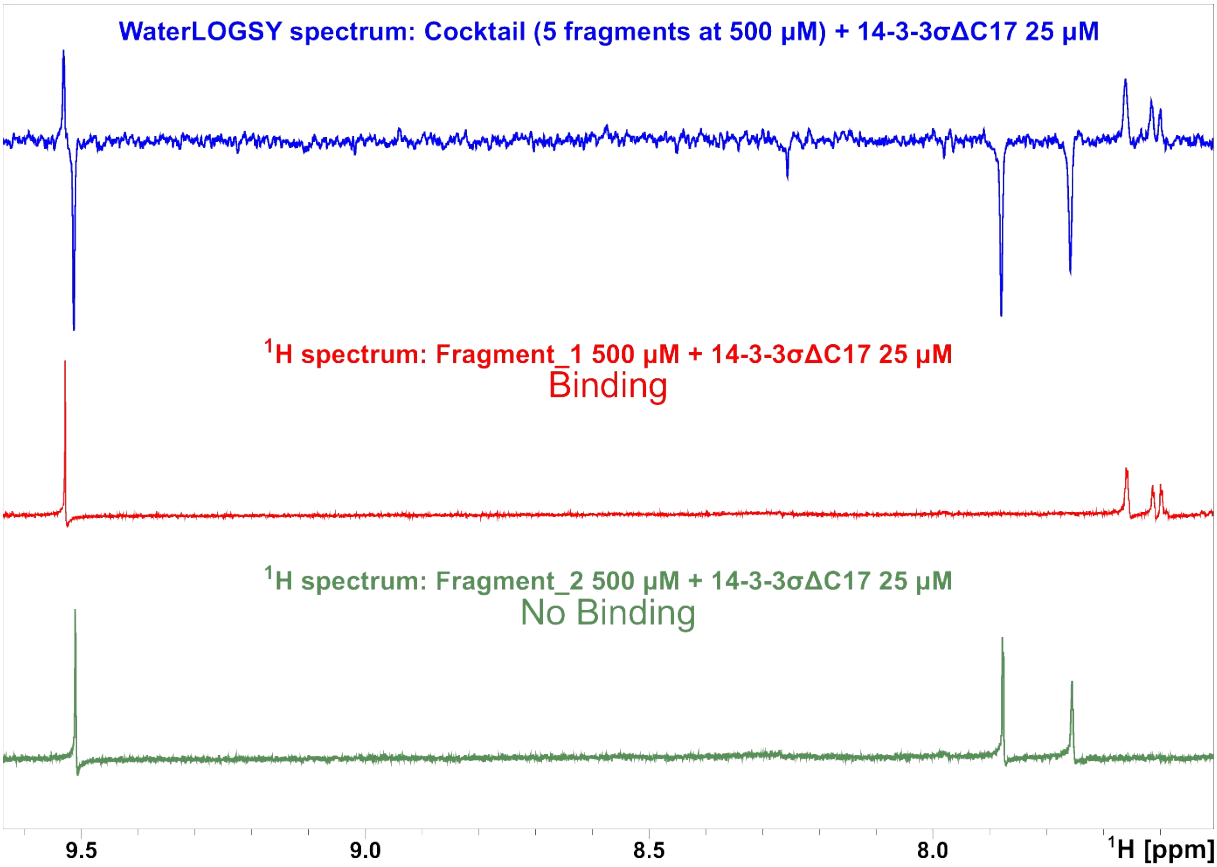


**Figure S4.** WaterLOGSY allows the determination of which singleton of the cocktail is binding to 14-3-3σΔC17. A WaterLOGSY spectrum of a Cocktail is shown at the top, in blue. A $^{1}$H spectrum of a hit singleton (Fragment_1) of the same cocktail is presented below the WaterLOGSY spectrum, in red. Note that the resonances of this molecule are positive in the WaterLOGSY spectrum, showing that this molecule binds to 14-3-3σΔC17. At the bottom, a $^{1}$H spectrum of a non-binder singleton (Fragment_2) is shown in green. Note that the resonances of this molecule are negative in the WaterLOGSY spectrum of the cocktail, meaning that this compound does not bind to 14-3-3σΔC17. The three spectra are referenced equally and were acquired in the same conditions.

**Table S1.** DSF melting temperature data for the screened cocktail library. The average melting temperatures, standard deviations and induced thermal shifts (ΔTm) are reported for each cocktail.

| COCKTAIL | Average Tm 1 (°C) n=3 | SD Tm 1 (°C) n=3 | ΔTm 1 (°C) | Average Tm 2 (°C) n=3 | SD Tm 2 (°C) n=3 | ΔTm 2 (°C) | ΔTm1 + ΔTm2 (°C) |
|---|---|---|---|---|---|---|---|
| **1** | 52,54 | 0,11 | -0,31 | 65,69 | 0,19 | -0,03 | **0,35** |
| **2** | 53,02 | 0,06 | 0,16 | 66,33 | 0,06 | 0,60 | **0,76** |
| **3** | 52,92 | 0,15 | 0,06 | 66,35 | 0,25 | 0,63 | **0,69** |
| **4** | 52,80 | 0,11 | -0,06 | 65,82 | 0,20 | 0,09 | **0,15** |
| **5** | 52,73 | 0,05 | -0,13 | 65,76 | 0,06 | 0,03 | **0,16** |
| **6** | 52,89 | 0,15 | 0,03 | 66,04 | 0,20 | 0,31 | **0,35** |
| **7** | 52,98 | 0,06 | 0,13 | 66,20 | 0,27 | 0,47 | **0,60** |
| **8** | 52,57 | 0,10 | -0,28 | 65,60 | 0,09 | -0,13 | **0,41** |
| **9** | 52,54 | 0,05 | -0,32 | 65,88 | 0,00 | 0,15 | **0,47** |
| **10** | 51,50 | 0,11 | -1,35 | 66,07 | 0,10 | 0,35 | **1,70** |
| **11** | - | - | - | - | - | - | **-** |
| **12** | 51,57 | 0,11 | -1,29 | 66,13 | 0,11 | 0,41 | **1,70** |
| **13** | 52,81 | 0,06 | -0,05 | 65,79 | 0,17 | 0,06 | **0,11** |
| **14** | 52,76 | 0,10 | -0,09 | 65,82 | 0,24 | 0,09 | **0,19** |
| **15** | 53,11 | 0,14 | 0,25 | 66,23 | 0,24 | 0,50 | **0,75** |
| **16** | 53,17 | 0,10 | 0,31 | 66,20 | 0,11 | 0,47 | **0,78** |
| **17** | 52,95 | 0,10 | 0,10 | 65,95 | 0,14 | 0,22 | **0,32** |
| **18** | 52,64 | 0,24 | -0,22 | 65,69 | 0,16 | -0,03 | **0,25** |
| **19** | 53,14 | 0,28 | 0,28 | 66,32 | 0,22 | 0,60 | **0,88** |
| **20** | 53,20 | 0,14 | 0,35 | 66,42 | 0,14 | 0,69 | **1,04** |
| **21** | 52,54 | 0,20 | -0,32 | 65,73 | 0,11 | 0,00 | **0,32** |
| **22** | 52,98 | 0,38 | 0,13 | 66,20 | 0,05 | 0,47 | **0,60** |
| **23** | 52,95 | 0,10 | 0,10 | 66,13 | 0,11 | 0,41 | **0,50** |
| **24** | 53,11 | 0,20 | 0,25 | 66,39 | 0,11 | 0,66 | **0,91** |
| **25** | 52,67 | 0,19 | -0,19 | 66,04 | 0,31 | 0,31 | **0,50** |
| **26** | 52,73 | 0,11 | -0,12 | 66,20 | 0,11 | 0,47 | **0,59** |
| **27** | 53,11 | 0,20 | 0,25 | 66,48 | 0,05 | 0,75 | **1,01** |
| **28** | 53,05 | 0,10 | 0,19 | 66,23 | 0,24 | 0,50 | **0,69** |
| **29** | 52,86 | 0,00 | 0,00 | 66,17 | 0,00 | 0,44 | **0,45** |
| **30** | 53,02 | 0,14 | 0,16 | 66,26 | 0,17 | 0,54 | **0,70** |
| **31** | 52,73 | 0,20 | 0,19 | 66,07 | 0,10 | -0,03 | **0,22** |
| **32** | 52,67 | 0,17 | 0,12 | 65,76 | 0,29 | -0,35 | **0,47** |
| **33** | 52,54 | 0,33 | 0,00 | 66,13 | 0,29 | 0,03 | **0,03** |
| **34** | 52,58 | 0,43 | 0,03 | 66,04 | 0,24 | -0,06 | **0,10** |
| **35** | 52,48 | 0,10 | -0,07 | 66,04 | 0,20 | -0,06 | **0,13** |
| **36** | 43,32 | 0,25 | -9,22 | 65,09 | 0,11 | -1,01 | **10,23** |
| **37** | 52,94 | 0,42 | 0,40 | 66,04 | 0,13 | -0,06 | **0,46** |
| **38** | 52,51 | 0,11 | -0,04 | 65,94 | 0,11 | -0,16 | **0,20** |
| **39** | 52,32 | 0,28 | -0,22 | 66,04 | 0,15 | -0,06 | **0,29** |
| **40** | 52,79 | 0,45 | 0,25 | 65,88 | 0,10 | -0,22 | **0,47** |

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|---|---|---|---|---|---|---|---|
| **41** | 52,83 | 0,06 | 0,28 | 65,69 | 0,16 | -0,41 | **0,69** |
| **42** | 52,67 | 0,00 | 0,13 | 66,17 | 0,00 | 0,07 | **0,19** |
| **43** | 52,56 | 0,36 | 0,02 | 66,10 | 0,06 | 0,02 | **0,19** |
| **44** | 52,48 | 0,43 | -0,07 | 65,82 | 0,24 | -0,28 | **0,35** |
| **45** | 52,13 | 0,49 | -0,41 | 66,20 | 0,43 | 0,09 | **0,50** |
| **46** | 52,60 | 0,24 | 0,06 | 66,04 | 0,11 | -0,06 | **0,12** |
| **47** | 51,82 | 0,33 | -0,72 | 65,69 | 0,10 | -0,41 | **1,13** |
| **48** | 52,58 | 0,34 | 0,03 | 65,91 | 0,14 | -0,19 | **0,22** |
| **49** | 52,42 | 0,38 | -0,13 | 65,72 | 0,14 | -0,38 | **0,51** |
| **50** | 52,20 | 0,29 | -0,35 | 65,88 | 0,17 | -0,22 | **0,57** |
| **51** | 51,25 | 0,34 | -1,29 | 65,28 | 0,11 | -0,82 | **2,11** |
| **52** | 52,51 | 0,33 | -0,03 | 65,76 | 0,29 | -0,35 | **0,38** |
| **53** | 52,60 | 0,24 | 0,06 | 65,98 | 0,10 | -0,13 | **0,19** |
| **54** | 52,67 | 0,41 | 0,13 | 66,01 | 0,11 | -0,10 | **0,22** |
| **55** | 52,48 | 0,28 | -0,06 | 65,91 | 0,14 | -0,19 | **0,25** |
| **56** | 52,32 | 0,14 | -0,22 | 65,72 | 0,06 | -0,38 | **0,60** |
| **57** | 52,98 | 0,43 | 0,44 | 65,60 | 0,25 | -0,50 | **0,94** |
| **58** | 52,95 | 0,10 | 0,41 | 65,98 | 0,00 | -0,12 | **0,53** |
| **59** | 52,86 | 0,10 | 0,31 | 65,88 | 0,17 | -0,22 | **0,53** |
| **60** | 52,86 | 0,10 | 0,31 | 65,76 | 0,14 | -0,35 | **0,66** |
| **61** | 52,80 | 0,29 | 0,28 | 65,85 | 0,24 | 0,00 | **0,28** |
| **62** | 52,76 | 0,34 | 0,25 | 66,09 | 0,32 | 0,24 | **0,49** |
| **63** | 53,08 | 0,29 | 0,56 | 66,07 | 0,10 | 0,22 | **0,79** |
| **64** | 51,66 | 0,14 | -0,85 | 65,60 | 0,09 | -0,25 | **1,10** |
| **65** | 52,45 | 0,14 | -0,07 | 65,95 | 0,14 | 0,10 | **0,16** |
| **66** | 52,57 | 0,10 | 0,06 | 65,91 | 0,07 | 0,06 | **0,12** |
| **67** | 51,82 | 0,48 | -0,69 | 65,88 | 0,10 | 0,03 | **0,73** |
| **68** | 52,70 | 0,05 | 0,19 | 66,01 | 0,05 | 0,16 | **0,35** |
| **69** | 52,54 | 0,20 | 0,03 | 65,76 | 0,14 | -0,09 | **0,12** |
| **70** | 52,92 | 0,11 | 0,41 | 66,42 | 0,14 | 0,57 | **0,98** |
| **71** | 52,57 | 0,20 | 0,06 | 65,79 | 0,10 | -0,06 | **0,12** |
| **72** | 52,54 | 0,15 | 0,03 | 65,79 | 0,10 | -0,06 | **0,09** |
| **73** | 50,40 | 0,10 | -2,11 | 65,26 | 0,11 | -0,59 | **2,70** |
| **74** | 51,03 | 0,11 | -1,48 | 66,67 | 0,15 | 0,82 | **2,30** |
| **75** | 52,26 | 0,05 | -0,25 | 65,88 | 0,10 | 0,03 | **0,29** |
| **76** | 52,83 | 0,14 | 0,31 | 65,88 | 0,10 | 0,03 | **0,35** |
| **77** | 52,57 | 0,17 | 0,06 | 65,79 | 0,25 | -0,06 | **0,12** |
| **78** | 52,57 | 0,10 | 0,06 | 66,01 | 0,11 | 0,16 | **0,22** |
| **79** | 52,64 | 0,06 | 0,12 | 65,91 | 0,06 | 0,06 | **0,19** |
| **80** | 52,67 | 0,00 | 0,16 | 65,88 | 0,17 | 0,03 | **0,19** |
| **81** | 52,73 | 0,20 | 0,22 | 66,01 | 0,11 | 0,16 | **0,37** |
| **82** | 53,14 | 0,00 | 0,63 | 66,23 | 0,28 | 0,38 | **1,01** |
| **83** | 52,83 | 0,14 | 0,31 | 65,85 | 0,15 | 0,00 | **0,31** |
| **84** | 52,10 | 0,10 | -0,41 | 66,42 | 0,38 | 0,57 | **0,98** |
| **85** | 52,95 | 0,28 | 0,44 | 66,48 | 0,20 | 0,63 | **1,07** |

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|---|---|---|---|---|---|---|---|
| **86** | 52,80 | 0,22 | 0,28 | 66,04 | 0,31 | 0,19 | **0,47** |
| **87** | 53,02 | 0,06 | 0,50 | 66,04 | 0,45 | 0,19 | **0,69** |
| **88** | 52,92 | 0,27 | 0,40 | 66,23 | 0,20 | 0,38 | **0,78** |
| **89** | 52,70 | 0,20 | 0,19 | 66,20 | 0,28 | 0,35 | **0,54** |
| **90** | 52,71 | 0,41 | 0,34 | 65,73 | 0,29 | 0,00 | **0,34** |
| **91** | 52,59 | 0,14 | 0,22 | 66,20 | 0,00 | 0,47 | **0,69** |
| **92** | 52,65 | 0,05 | 0,28 | 66,26 | 0,15 | 0,53 | **0,82** |
| **93** | 52,43 | 0,09 | 0,06 | 65,69 | 0,11 | -0,03 | **0,10** |
| **94** | 52,40 | 0,14 | 0,03 | 65,79 | 0,05 | 0,06 | **0,09** |
| **95** | 51,93 | 0,21 | -0,44 | 65,88 | 0,14 | 0,16 | **0,60** |
| **96** | 52,49 | 0,05 | 0,12 | 66,32 | 0,07 | 0,60 | **0,72** |
| **97** | 52,40 | 0,05 | 0,03 | 65,82 | 0,09 | 0,09 | **0,13** |
| **98** | 52,24 | 0,17 | -0,12 | 65,91 | 0,10 | 0,19 | **0,31** |
| **99** | 52,40 | 0,05 | 0,03 | 65,91 | 0,32 | 0,19 | **0,22** |
| **100** | 52,81 | 0,00 | 0,44 | 66,16 | 0,11 | 0,44 | **0,88** |
| **101** | 51,87 | 0,11 | -0,50 | 65,88 | 0,19 | 0,16 | **0,66** |
| **102** | 52,52 | 0,16 | 0,16 | 65,82 | 0,09 | 0,09 | **0,25** |
| **103** | 52,68 | 0,28 | 0,31 | 66,13 | 0,33 | 0,41 | **0,72** |
| **104** | 52,52 | 0,16 | 0,16 | 66,20 | 0,41 | 0,47 | **0,63** |
| **105** | 52,46 | 0,10 | 0,09 | 65,91 | 0,19 | 0,19 | **0,28** |
| **106** | 52,40 | 0,05 | 0,03 | 66,10 | 0,19 | 0,37 | **0,41** |
| **107** | 48,60 | 0,30 | -3,77 | 66,29 | 0,17 | 0,57 | **4,34** |
| **108** | 52,52 | 0,10 | 0,16 | 66,51 | 0,05 | 0,78 | **0,94** |
| **109** | 52,49 | 0,05 | 0,12 | 66,26 | 0,05 | 0,53 | **0,66** |
| **110** | 52,46 | 0,10 | 0,09 | 65,88 | 0,05 | 0,15 | **0,25** |
| **111** | 52,43 | 0,25 | 0,06 | 66,32 | 0,20 | 0,60 | **0,66** |
| **112** | 52,49 | 0,11 | 0,13 | 66,16 | 0,11 | 0,44 | **0,56** |
| **113** | 52,49 | 0,14 | 0,13 | 66,13 | 0,24 | 0,41 | **0,53** |
| **114** | 52,43 | 0,09 | 0,06 | 66,17 | 0,06 | 0,44 | **0,50** |
| **115** | 51,74 | 0,14 | -0,63 | 66,10 | 0,10 | 0,38 | **1,01** |
| **116** | 52,81 | 0,00 | 0,44 | 66,45 | 0,20 | 0,72 | **1,17** |
| **117** | 52,40 | 0,20 | 0,03 | 66,54 | 0,36 | 0,82 | **0,85** |
| **118** | 52,71 | 0,34 | 0,35 | 66,32 | 0,14 | 0,60 | **0,94** |
| **119** | 52,52 | 0,10 | 0,16 | 66,54 | 0,05 | 0,81 | **0,97** |
| **120** | 52,57 | 0,17 | 0,10 | 66,04 | 0,15 | 0,28 | **0,38** |
| **121** | 52,48 | 0,17 | 0,00 | 66,20 | 0,20 | 0,44 | **0,44** |
| **122** | 52,60 | 0,14 | 0,13 | 66,07 | 0,10 | 0,32 | **0,44** |
| **123** | 50,84 | 0,19 | -1,63 | 65,22 | 0,16 | -0,53 | **2,17** |
| **124** | 48,54 | 0,45 | -3,94 | 65,22 | 0,25 | -0,53 | **4,47** |
| **125** | 52,48 | 0,10 | 0,00 | 65,92 | 0,11 | 0,16 | **0,16** |
| **126** | 52,64 | 0,14 | 0,16 | 66,39 | 0,39 | 0,64 | **0,80** |
| **127** | 51,19 | 0,11 | -1,29 | 65,82 | 0,11 | 0,06 | **1,35** |
| **128** | 52,42 | 0,11 | -0,06 | 65,82 | 0,05 | 0,06 | **0,12** |
| **129** | 52,42 | 0,24 | -0,06 | 66,04 | 0,34 | 0,28 | **0,34** |
| **130** | 50,50 | 0,49 | -1,98 | 63,87 | 0,43 | -1,89 | **3,87** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **131** | 52,29 | 0,09 | -0,19 | 65,57 | 0,14 | -0,19 | **0,38** |
| **132** | 52,48 | 0,00 | 0,00 | 65,63 | 0,10 | -0,13 | **0,13** |
| **133** | 52,51 | 0,11 | 0,03 | 66,04 | 0,20 | 0,28 | **0,31** |
| **134** | 52,70 | 0,22 | 0,22 | 66,35 | 0,41 | 0,60 | **0,82** |
| **135** | 52,35 | 0,05 | -0,13 | 65,75 | 0,11 | 0,00 | **0,13** |
| **136** | 52,73 | 0,05 | 0,25 | 66,17 | 0,16 | 0,41 | **0,66** |
| **137** | 52,63 | 0,29 | 0,16 | 66,48 | 0,14 | 0,73 | **0,88** |
| **138** | 48,76 | 0,11 | -3,71 | 66,83 | 0,00 | 1,07 | **4,79** |
| **139** | 52,54 | 0,15 | 0,06 | 65,85 | 0,22 | 0,10 | **0,16** |
| **140** | 52,29 | 0,00 | -0,19 | 65,85 | 0,33 | 0,10 | **0,28** |
| **141** | 52,67 | 0,10 | 0,19 | 66,48 | 0,14 | 0,73 | **0,92** |
| **142** | 52,57 | 0,19 | 0,09 | 66,11 | 0,22 | 0,35 | **0,44** |
| **143** | 52,45 | 0,06 | -0,03 | 65,76 | 0,06 | 0,00 | **0,03** |
| **144** | 52,79 | 0,36 | 0,32 | 66,32 | 0,24 | 0,57 | **0,88** |
| **145** | 52,54 | 0,05 | 0,06 | 66,26 | 0,17 | 0,51 | **0,57** |
| **146** | 52,20 | 0,10 | -0,28 | 65,60 | 0,09 | -0,16 | **0,44** |
| **147** | 50,25 | 0,11 | -2,23 | 65,98 | 0,19 | 0,22 | **2,45** |
| **148** | 52,35 | 0,14 | -0,12 | 65,98 | 0,35 | 0,22 | **0,34** |
| **149** | 48,13 | 0,02 | -4,01 | 66,45 | 0,09 | 0,60 | **4,61** |
| **150** | 51,54 | 0,32 | -0,60 | 66,14 | 0,06 | 0,29 | **0,89** |
| **151** | 52,22 | 0,46 | 0,09 | 66,45 | 0,34 | 0,60 | **0,68** |
| **152** | 51,94 | 0,20 | -0,19 | 65,66 | 0,19 | -0,19 | **0,38** |
| **153** | 51,79 | 0,36 | -0,35 | 65,79 | 0,10 | -0,06 | **0,41** |
| **154** | 52,10 | 0,00 | -0,04 | 65,85 | 0,05 | 0,00 | **0,04** |
| **155** | 52,14 | 0,11 | 0,00 | 66,18 | 0,10 | 0,33 | **0,33** |
| **156** | 52,24 | 0,05 | 0,10 | 65,92 | 0,11 | 0,07 | **0,17** |
| **157** | 52,20 | 0,00 | 0,06 | 65,82 | 0,05 | -0,03 | **0,09** |

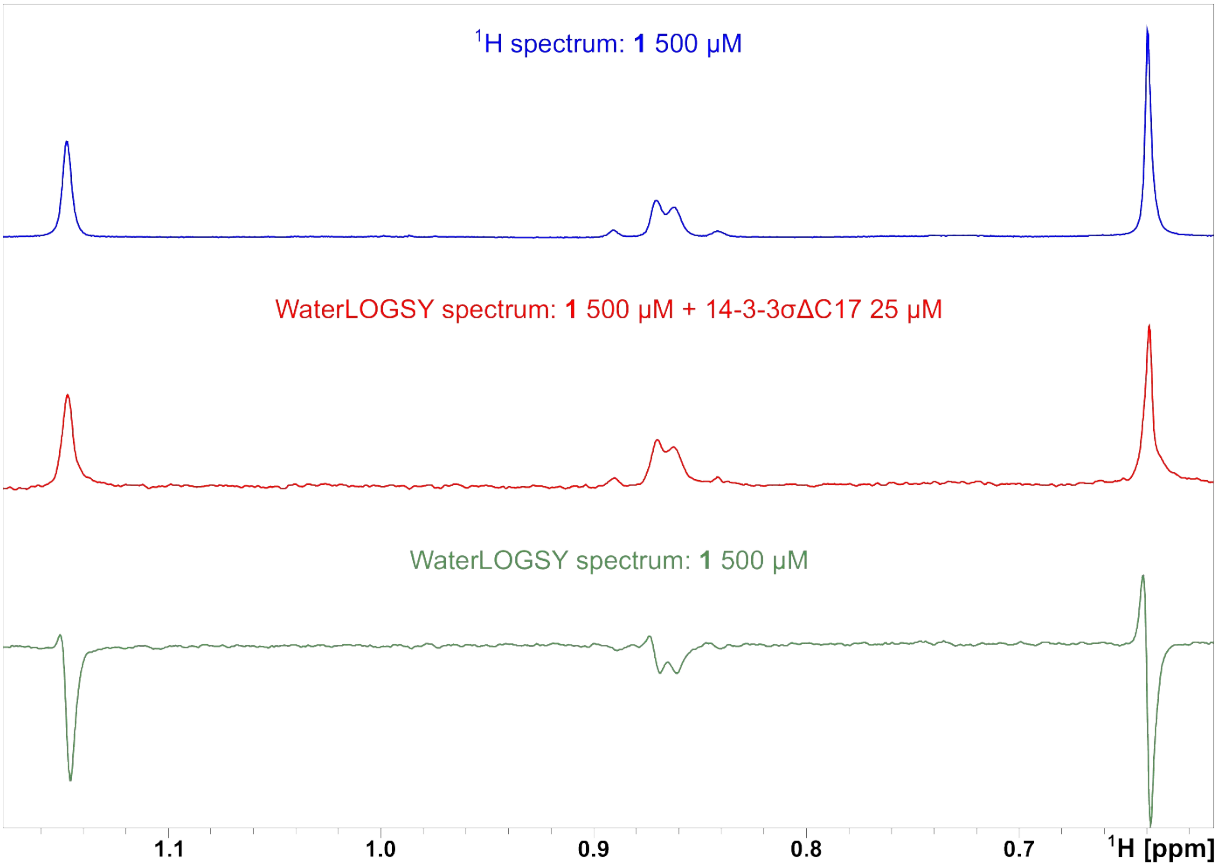


**Figure S5.** WaterLOGSY detects the binding of **1** to 14-3-3σΔC17. $^1$H spectrum of **1** 500 µM (blue spectrum, on top). The WaterLOGSY spectrum of a solution containing **1** 500 µM and 14-3-3σΔC17 25 µM (in red) shows that the NMR signals of **1** are phased positive, indicating binding. The control WaterLOGSY spectrum of a solution containing **1** 500 µM alone (in green) shows that in the absence of 14-3-3σΔC17 the NMR signals of **1** 500 µM are all phased negative.

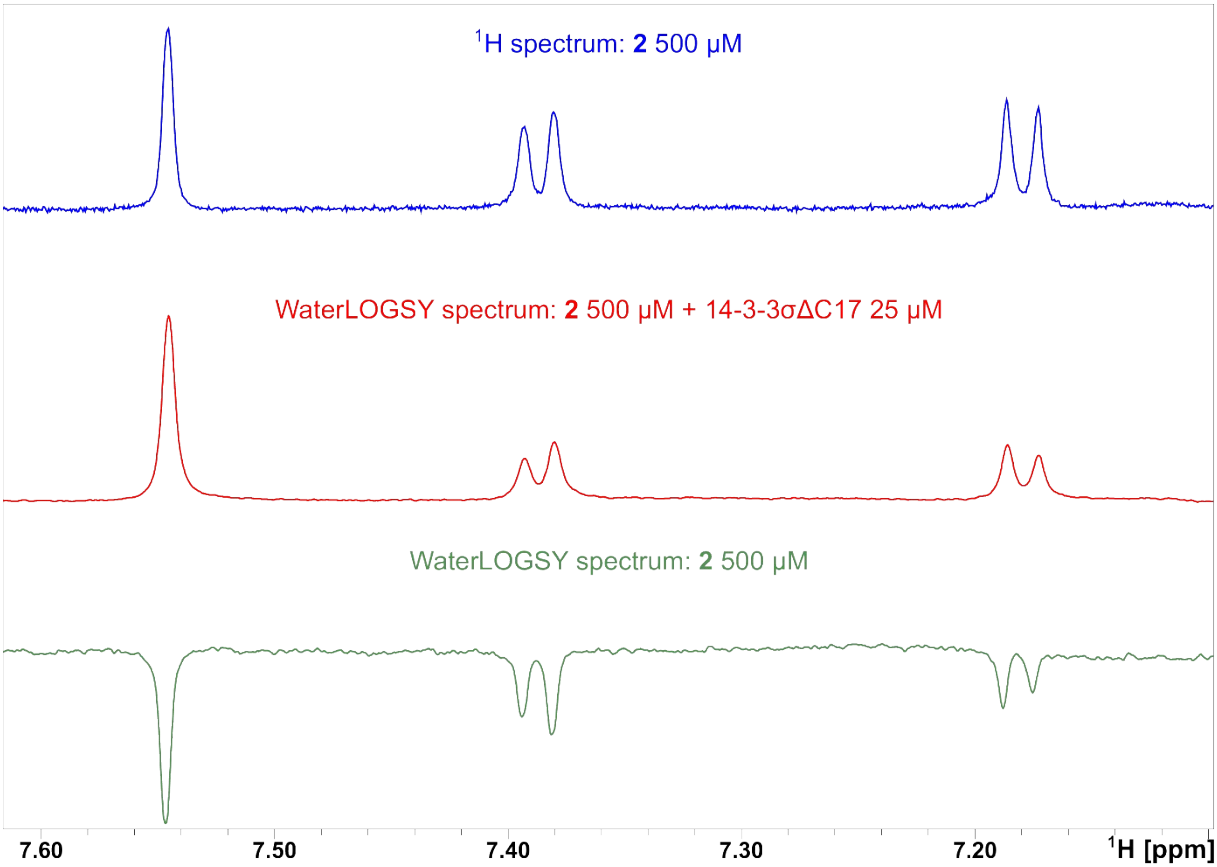


**Figure S6.** WaterLOGSY detects the binding of **2** to 14-3-3σΔC17. $^1$H spectrum of **2** 500 µM (blue spectrum, on top). The WaterLOGSY spectrum of a solution containing **2** 500 µM and 14-3-3σΔC17 25 µM (in red) shows that the NMR signals of **2** are phased positive, indicating binding. The control WaterLOGSY spectrum of a solution containing **2** 500 µM alone (in green) shows that in the absence of 14-3-3σΔC17 the NMR signals of **2** 500 µM are all phased negative.

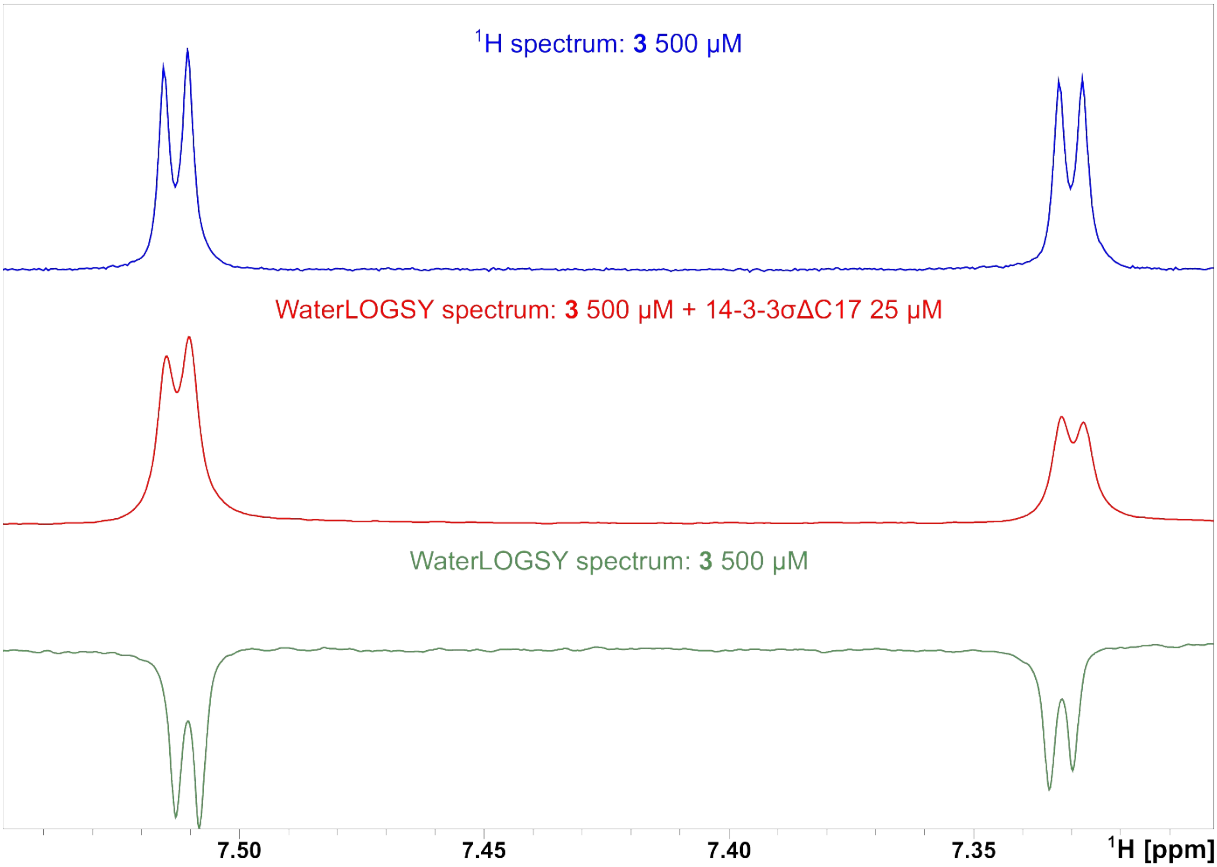


**Figure S7.** WaterLOGSY detects the binding of **3** to 14-3-3σΔC17. $^1$H spectrum of **3** 500 µM (blue spectrum, on top). The WaterLOGSY spectrum of a solution containing **3** 500 µM and 14-3-3σΔC17 25 µM (in red) shows that the NMR signals of **3** are phased positive, indicating binding. The control WaterLOGSY spectrum of a solution containing **3** 500 µM alone (in green) shows that in the absence of 14-3-3σΔC17 the NMR signals of **3** 500 µM are all phased negative.

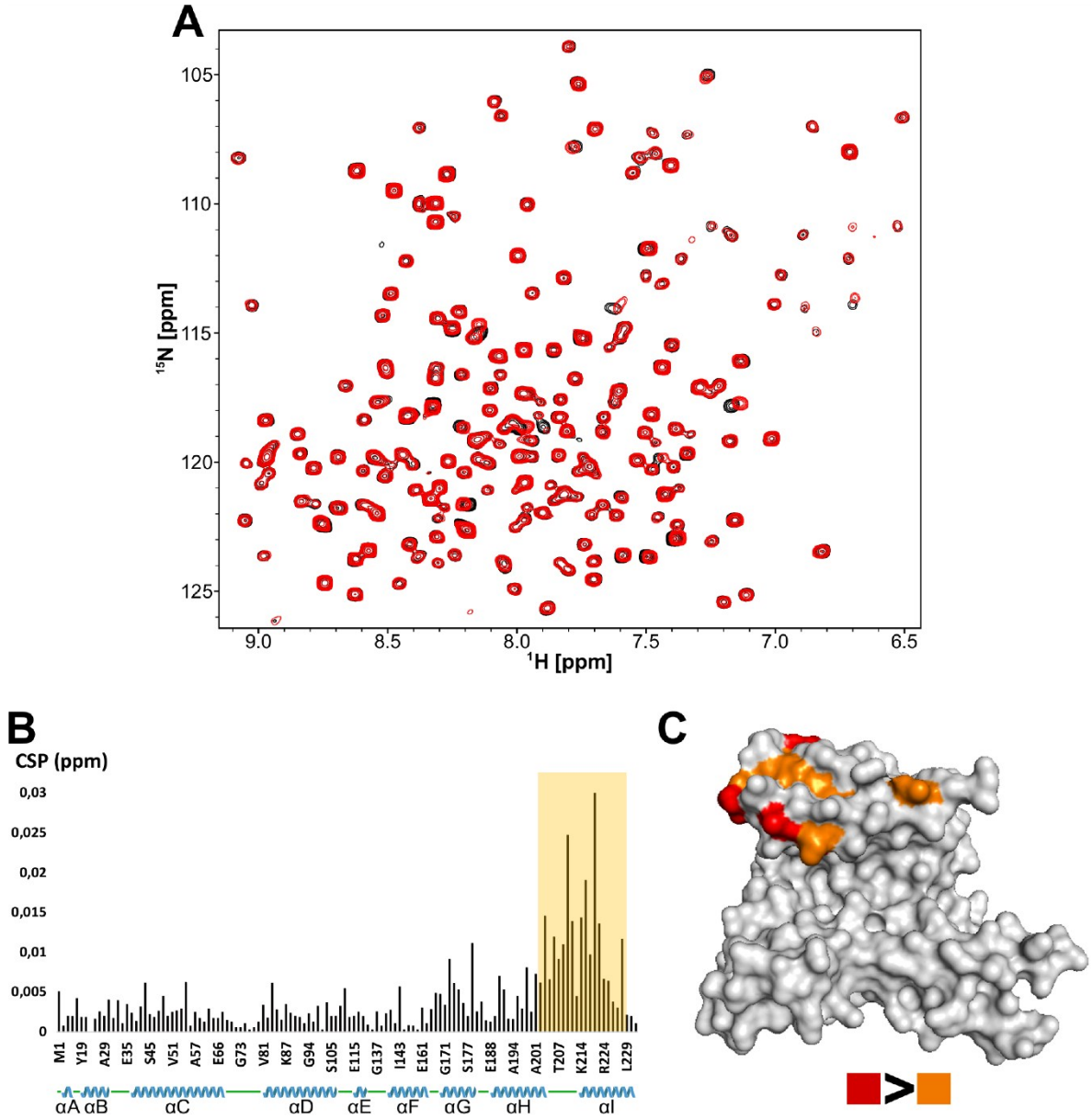


**Figure S8. 1** binding site on 14-3-3σΔC17 identified by $^{1}$H-$^{15}$N TROSY-HSQC. (A) Superimposed $^{1}$H-$^{15}$N TROSY-HSQC spectra of $^{15}$N$^{2}$H labelled 14-3-3σΔC17 75 µM in the presence of 2% DMSO-$d_6$ (v/v) (in black) and **1** 2000 µM (in red). (B) Plot of the CSP values (in ppm) of $^{1}$H-$^{15}$N correlation peaks of 75 µM 14-3-3σΔC17 in the presence of 2000 µM **1** compared to the reference 14-3-3σΔC17 spectrum (75 µM) (*y axis*) versus the amino acid sequence (*x axis*). A total of 128 correlation peak CSP are shown. The *x axis* is not proportional. The helices of 14-3-3σ are identified below the *x axis* as blue cartoons, while disordered regions are represented by green lines. The area highlighted in yellow shows the region of the protein affected by the presence of **1**. (C) Mapping on the crystal structure of 14-3-3σ (PDB ID: 1YZ5) of the amino acid residues corresponding to the 10 most affected resonances by the presence of **1**. The residues corresponding to the 5 most affected resonances are colored in red and an additional 5 are colored in orange.

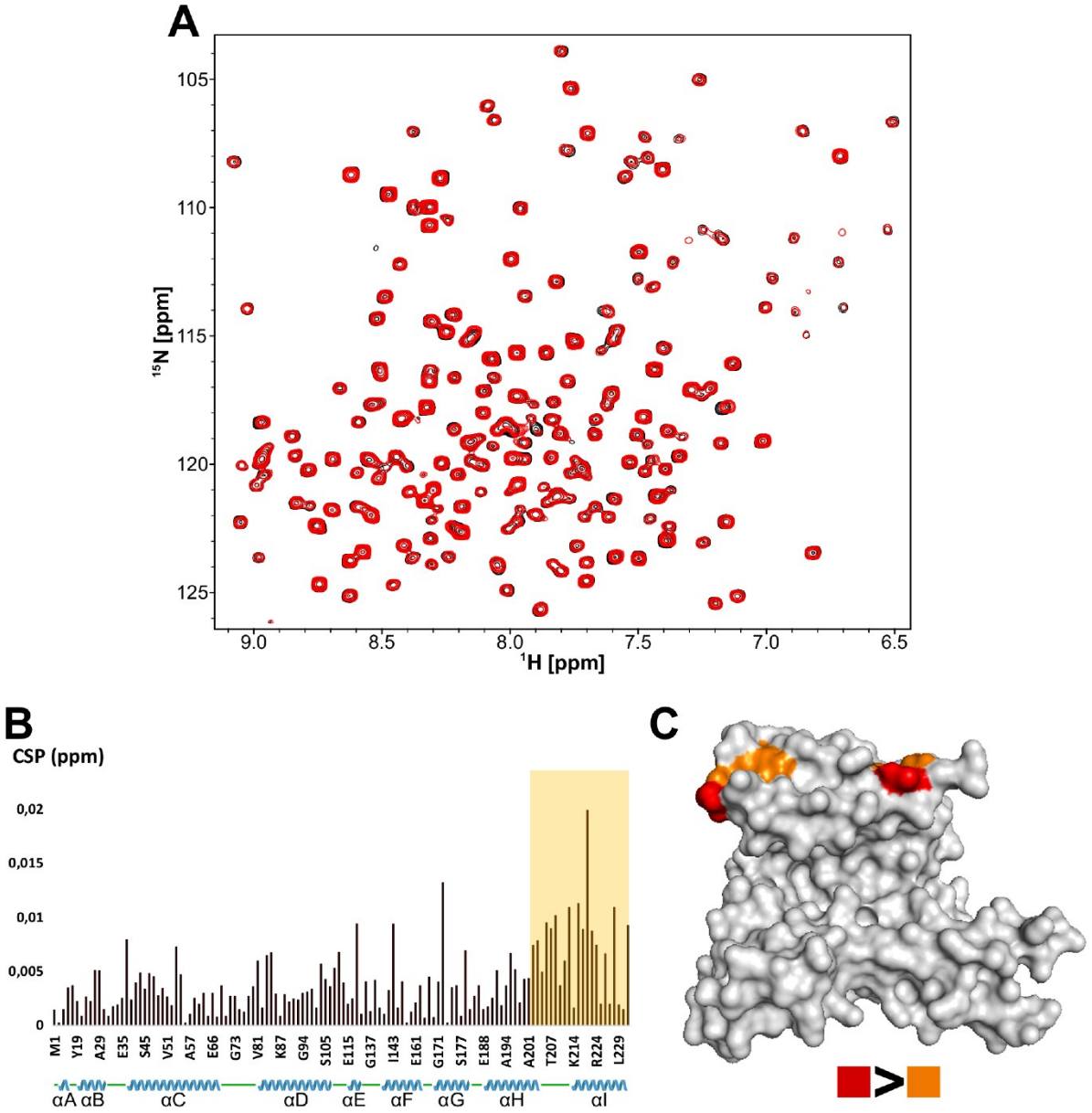


**Figure S9. 2** binding site on 14-3-3σΔC17 identified by $^{1}$H-$^{15}$N TROSY-HSQC. (A) Superimposed $^{1}$H-$^{15}$N TROSY-HSQC spectra of $^{15}$N$^{2}$H labelled 14-3-3σΔC17 75 µM in the presence of 2% DMSO-$d_6$ (v/v) (in black) and **2** 2000 µM (in red). (B) Plot of the CSP values (in ppm) of $^{1}$H-$^{15}$N correlation peaks of 75 µM 14-3-3σΔC17 in the presence of 2000 µM **2** compared to the reference 14-3-3σΔC17 spectrum (75 µM) (*y axis*) versus the amino acid sequence (*x axis*). A total of 128 correlation peak CSP are shown. The *x axis* is not proportional. The helices of 14-3-3σ are identified below the *x axis* as blue cartoons, while disordered regions are represented by green lines. The area highlighted in yellow shows the region of the protein affected by the presence of **2**. (C) Mapping on the crystal structure of 14-3-3σ (PDB ID: 1YZ5) of the amino acid residues corresponding to the 10 most affected resonances by the presence of **2**. The residues corresponding to the 5 most affected resonances are colored in red and an additional 5 are colored in orange.

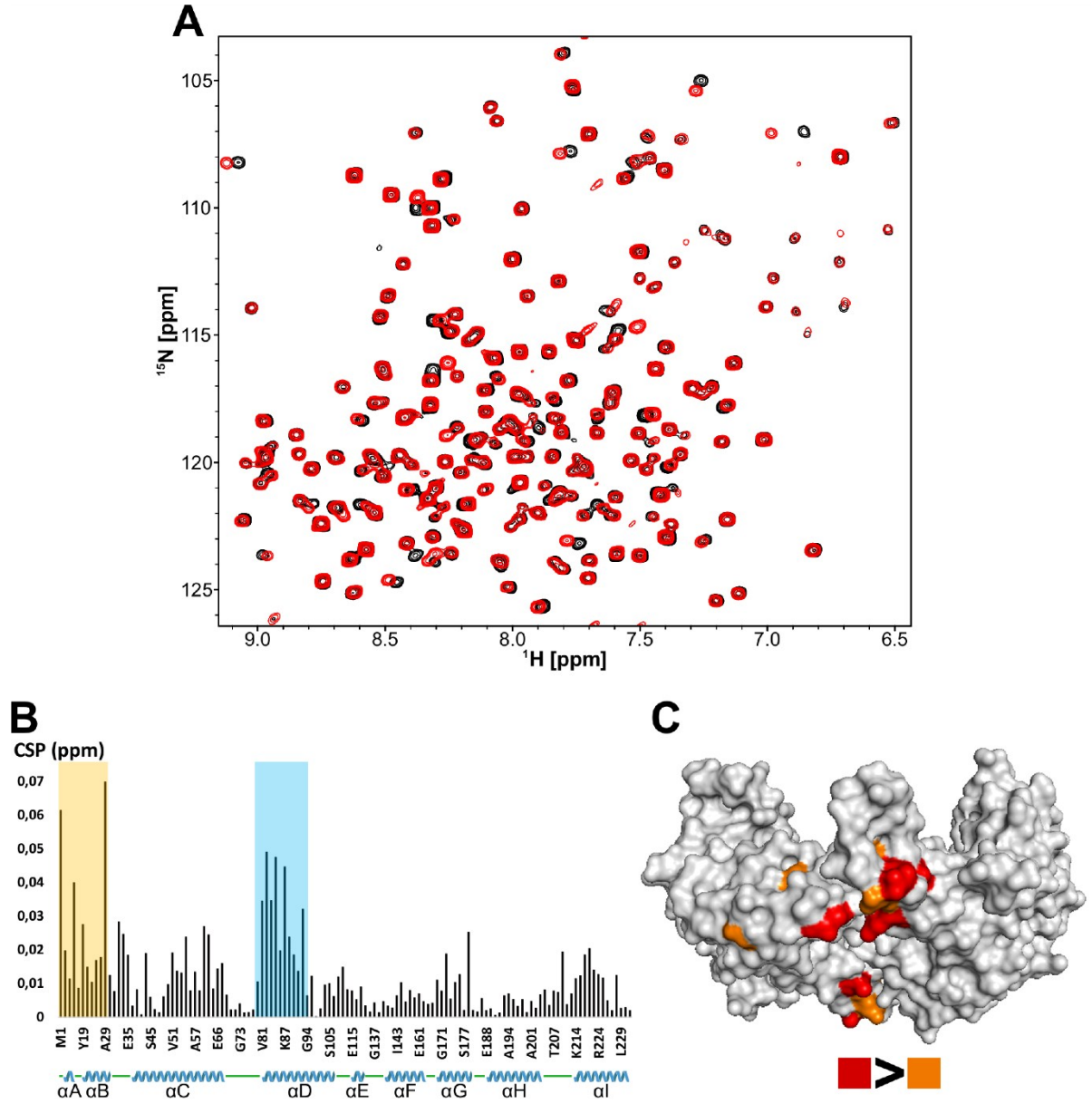


**Figure S10. 3** binding site on 14-3-3σΔC17 identified by $^{1}$H-$^{15}$N TROSY-HSQC. (A) Superimposed $^{1}$H-$^{15}$N TROSY-HSQC spectra of $^{15}$N$^{2}$H labelled 14-3-3σΔC17 75 µM in the presence of 2% DMSO-$d_6$ (v/v) (in black) and **3** 2000 µM (in red). (B) Plot of the CSP values (in ppm) of $^{1}$H-$^{15}$N correlation peaks of 75 µM 14-3-3σΔC17 in the presence of 2000 µM **3** compared to the reference 14-3-3σΔC17 spectrum (75 µM) (*y axis*) versus the amino acid sequence (*x axis*). A total of 128 correlation peak CSP are shown. The *x axis* is not proportional. The helices of 14-3-3σ are identified below the *x axis* as blue cartoons, while disordered regions are represented by green lines. The areas highlighted in yellow and blue show the region of the protein affected by the presence of **3**. (C) Mapping on the crystal structure of 14-3-3σ (PDB ID: 1YZ5) of the amino acid residues corresponding to the 10 most affected resonances by the presence of **3**. The residues corresponding to the 5 most affected resonances are colored in red and an additional 5 are colored in orange.