# Particle_Analysis_for_Excel

July 29, 2025

## 0.1 Particle Analysis

### 0.1.1 Automatic calculations & graphing

**Code Last updated on 2025-07-15 by Yinglin Li**

**(This is meant for protease activation events, otherwise the signmoid fitting is going to be wonky)**

**(Also this version needs the XY file to work.)**

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*7/14/25 - wrote code 7/15/25 - redid name outputs for excel + plots to include input file name 7/28/25 - fixed a bug with sigmoid plot fitting and added a log file input to pull out parameters 7/29/25 - changed sigmoid function to one used in Sigmaplot and updated bleedthrough coefficients to one saquired in live cell settings*

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### 0.1.2 Set user defined parameters

**This is the *only* section of code that you need to modify.**

[268]:
```
# Set filepaths
intensity_file = r"C:\Users\yli355\Downloads\example of good particle\1ROI20I.
↪xlsx"
XY_file = r"C:\Users\yli355\Downloads\example of good particle\1ROI20xy.xls"
Log_file = r"C:\Users\yli355\Downloads\example of good particle\TL01_1_R3D.dv.
↪log"

# nFRET Coefficients
YFP_bleedthrough = 0.0714
mCH_bleedthrough = 0.0581

# Sigmoid fitting time (set to None to use min or max possible value)
lower_bound_in_minutes = None
upper_bound_in_minutes = None
```

### 0.1.3 *Do not modify* anything below this line

[269]:
```python
import pandas as pd
import numpy as np
import os
import re
from scipy.optimize import curve_fit
from sklearn.metrics import mean_squared_error
import matplotlib.pyplot as plt
from matplotlib.collections import LineCollection
from matplotlib import cm
from mpl_toolkits.axes_grid1 import make_axes_locatable

# read in files
df1 = pd.read_excel(intensity_file, sheet_name=0, header=0)
df2 = pd.read_excel(intensity_file, sheet_name=6, header=0)
df3 = pd.read_excel(intensity_file, sheet_name=12, header=0)
xy = pd.read_excel(XY_file, skiprows=2)
log = open(Log_file).readlines()

# Get name of the file (using the intensity file, or whatever is filepath1
base_name = os.path.splitext(os.path.basename(intensity_file))[0]

# Make output file names
summary_filename = f"{base_name}_Summary.xlsx"
nFRET_fit_img = f"{base_name}_nFRET_Fit.png"
CB_fit_img = f"{base_name}_CB_Fit.png"
trajectory_img = f"{base_name}_trajectory.png"
intensity_img = f"{base_name}_intensity.png"

# Make a folder for the outputs
filepath_out = os.path.splitext(intensity_file)[0]
os.makedirs(filepath_out, exist_ok=True)

# Get parameters from log file
pixel_line = next(line for line in log if "Pixel Size" in line)
match = re.search(r"[\d.]+", pixel_line)
Dim = float(match.group()) if match else None

temporal_resolution_line = next(line for line in log if "TLAPSE" in line)
match = re.search(r"[\d.]+", temporal_resolution_line)
Tempres = float(match.group()) if match else None

filter_lines = [line.strip() for line in log if line.strip().
 ↪startswith("FILTERS")]
channel_map = {}
for line in filter_lines:
```

```python
    if "488Laser,GFP" in line:
        channel_map['YFP'] = line
    elif "488Laser,mCherry" in line:
        channel_map['FRET'] = line
    elif "561Laser,mCherry" in line:
        channel_map['mCherry'] = line
ordered_labels = []
for line in filter_lines:
    for label, value in channel_map.items():
        if line == value:
            ordered_labels.append(label)
channel_numbers = {label: i + 1 for i, label in enumerate(ordered_labels)}
YFP = channel_numbers['YFP']
FRET = channel_numbers['FRET']
mCH = channel_numbers['mCherry']

def correct_shift(df):
    """
    Function to align the values into a single column for when the final track␣
 ↪is stitched together from multiple tracks.
    Shifts all columns except the first column (the column with frame numbers)␣
 ↪to the left, aligning each row individually.

    After that, shifts all rows up by one.
    Finally, drops any columns that are all NaN.

    Parameters:
    - df (pd.DataFrame): The dataframe to be shifted

    Returns:
    - pd.DataFrame: DataFrame with left-shifted values (NaNs on the right),␣
 ↪rows shifted up,
                    and all-NaN columns dropped
    """
    index_col = 0  # fixed index column

    # Extract the frame number column
    preserved_col = df.iloc[:, index_col]
    data_to_shift = df.drop(df.columns[index_col], axis=1)

    # Get shift value for rows
    n_shiftup = preserved_col.count() - df.shape[0]

    # Function to left-align a single row
    def left_align_row(row):
        non_nan_values = row.dropna().tolist()
        n_nans = len(row) - len(non_nan_values)
```

```
        return pd.Series(non_nan_values + [np.nan]*n_nans, index=row.index)

    # Apply left alignment row-wise
    shifted_data = data_to_shift.apply(left_align_row, axis=1)

    # Combine back with preserved column
    result_df = pd.concat([preserved_col, shifted_data], axis=1)

    # Set preserved column as index
    result_df.set_index(result_df.columns[0], inplace=True)

    # Shift up (drop first row, reindex) but keep all rows
    result_df = result_df.shift(n_shiftup)

    # Drop columns and rows that are all NaN
    result_df = result_df.dropna(axis=1, how='all')
    result_df = result_df.dropna(axis=0, how='all')

    # Re-index rows
    result_df = result_df.reset_index(drop=True)

    return result_df

def rename_channels(df, YFP, FRET, mCH):
    """
    Renames the columns to 'YFP', 'FRET', and 'mCH' based on user-defined␣
 ↪channel numbers.

    Parameters:
    - df (pd.DataFrame): DataFrame with at least 3 data columns (ignoring index)
    - YFP, FRET, mCH (int): Numbers assigned to each channel

    Returns:
    - pd.DataFrame: DataFrame with renamed columns
    """
    # Standardize the column names to A, B, C for easy mapping later on
    df.columns = ['A', 'B', 'C']

    # Build map from names based on position
    channel_labels = {YFP: 'YFP', FRET: 'FRET', mCH: 'mCH'}

    #Create list of new column names
    new_column_names = [channel_labels[i + 1] for i in range(3)]

    #Assign new column names
    df.columns = new_column_names
```

```python
    return df

def sigmoid(x, y0, a, x0, b):
    return y0 + a / (1 + np.exp(-(x - x0) / b))

def fit_and_report(df, x_col, y_col, lower_bound_in_minutes=None, upper_bound_in_minutes=None):
    x_data_all = df[x_col].values
    y_data_all = df[y_col].values

    # Set fit range limits if None
    if lower_bound_in_minutes is None:
        lower_bound_in_minutes = np.min(x_data_all)
    if upper_bound_in_minutes is None:
        upper_bound_in_minutes = np.max(x_data_all)

    # Select data within fit range
    mask = (x_data_all >= lower_bound_in_minutes) & (x_data_all <= upper_bound_in_minutes)
    x_data = x_data_all[mask]
    y_data = y_data_all[mask]

    if len(x_data) == 0:
        raise ValueError("No data points found in fit range. Check lower_bound_in_minutes and upper_bound_in_minutes.")

    # Initial guess: L, k, x0, b
    initial_guess = [max(y_data) - min(y_data), 1, np.median(x_data), min(y_data)]

    # Allow k to be negative for decreasing sigmoid
    bounds = (
        [0, -5, min(x_data), min(y_data) - 0.5 * abs(min(y_data))],
        [1.5 * (max(y_data) - min(y_data)), 5, max(x_data), max(y_data) + 0.5 * abs(max(y_data))]
    )

    try:
        popt, _ = curve_fit(sigmoid, x_data, y_data, p0=initial_guess, bounds=bounds)
        L, k, x0, b = popt

        # Predict over full x range
        y_pred_all = sigmoid(x_data_all, *popt)
        residuals_all = y_data_all - y_pred_all
        mse = mean_squared_error(y_data, sigmoid(x_data, *popt))
```

```python
        def calc_x_for_y(y_target):
            return x0 - (1 / k) * np.log((L / (y_target - b)) - 1)

        ec10 = calc_x_for_y(b + 0.1 * L)
        ec90 = calc_x_for_y(b + 0.9 * L)
        pr_duration = abs(ec90 - ec10)

        print(f"\n< {y_col} >")
        print(f"Fitting range: {lower_bound_in_minutes} to␣
↪{upper_bound_in_minutes}")
        print(f"PR duration (ec90 - ec10): {pr_duration:.3f}")
        print(f"Mean Squared Error (fit range): {mse:.5f}\n")

    except Exception as e:
        print(f"\n< {y_col} >")
        print(f"Sigmoid fit failed for range {lower_bound_in_minutes} to␣
↪{upper_bound_in_minutes}: {e}")
        print("Filling predicted/residuals with NaN.\n")

        y_pred_all = np.full_like(x_data_all, np.nan)
        residuals_all = np.full_like(x_data_all, np.nan)
        ec10 = ec90 = pr_duration = mse = np.nan

    results_df = pd.DataFrame({
        x_col: x_data_all,
        'Predicted': y_pred_all,
        'Residual': residuals_all
    })

    metrics_df = pd.DataFrame({
        'ec10': [ec10],
        'ec90': [ec90],
        'PR duration': [pr_duration],
        'MSE': [mse]
    })

    return results_df, metrics_df

# Clean XY file as needed
xy = xy[[col for col in xy.columns if col in ['x', 'y']]]
xy = xy.dropna(how='all', subset=['x', 'y'])
xy = xy.reset_index(drop=True)

df = pd.concat([correct_shift(d) for d in [df1, df2, df3]], axis=1)

df = rename_channels(df, YFP, FRET, mCH)
```

```
df = pd.concat([df,xy],axis=1)

df['Time (sec)'] = df.index * Tempres
df['Time (min)'] = df.index * Tempres / 60
df['nFRET'] = (df['FRET'] - (YFP_bleedthrough * df['YFP']) - (mCH_bleedthrough␣
 ↪* df['mCH'])) / df['mCH']
df['CB'] = df['YFP'] / (df['YFP'] + df['mCH'])
df['x (um)'] = df['x'] * Dim
df['y (um)'] = df['y'] * Dim

df = df.iloc[:, [5,6,3,4,9,10,0,2,1,7,8]]

results_nFRET, metrics_nFRET = fit_and_report(df, 'Time (min)', 'nFRET',␣
 ↪lower_bound_in_minutes=lower_bound_in_minutes,␣
 ↪upper_bound_in_minutes=upper_bound_in_minutes)
results_CB, metrics_CB = fit_and_report(df, 'Time (min)', 'CB',␣
 ↪lower_bound_in_minutes=lower_bound_in_minutes,␣
 ↪upper_bound_in_minutes=upper_bound_in_minutes)
# Note: You want the lowest mean squared error possible

columns_to_drop = [
    'predicted nFRET', 'residual nFRET',
    'predicted CB', 'residual CB'
]

df = df.drop(columns=[col for col in columns_to_drop if col in df.columns],␣
 ↪errors='ignore')

results_nFRET = results_nFRET.drop(columns=['Time (min)'], errors='ignore')
results_nFRET = results_nFRET.rename(columns={
    'Predicted': 'predicted nFRET',
    'Residual': 'residual nFRET'
})

results_CB = results_CB.drop(columns=['Time (min)'], errors='ignore')
results_CB = results_CB.rename(columns={
    'Predicted': 'predicted CB',
    'Residual': 'residual CB'
})

df = pd.concat([df, results_nFRET, results_CB], axis=1)

metrics_nFRET.index = ['nFRET']
metrics_CB.index = ['CB']
kinetics = pd.concat([metrics_nFRET, metrics_CB])
```

```python
# Save output
filepath = os.path.join(filepath_out, f"{base_name}_Summary.xlsx")

with pd.ExcelWriter(filepath) as writer:
    df.to_excel(writer, sheet_name='Summary', index=True)
    kinetics.to_excel(writer, sheet_name='Kinetics', index=True)
```

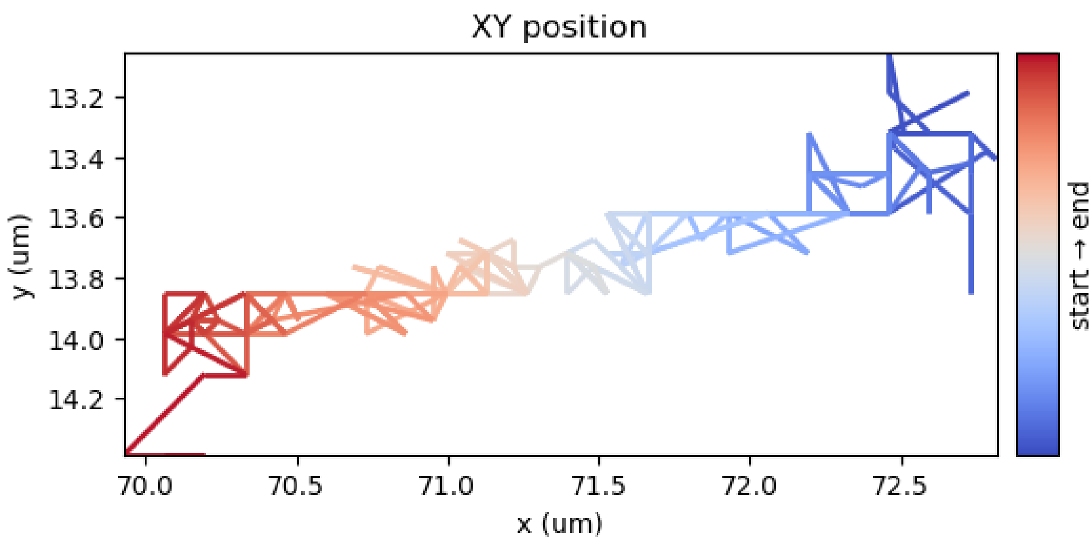
```
< nFRET >
Fitting range: 0.0 to 68.25
PR duration (ec90 - ec10): 13.210
Mean Squared Error (fit range): 0.02784


< CB >
Fitting range: 0.0 to 68.25
PR duration (ec90 - ec10): 20.540
Mean Squared Error (fit range): 0.00391
```

**Graphs**

[270]:

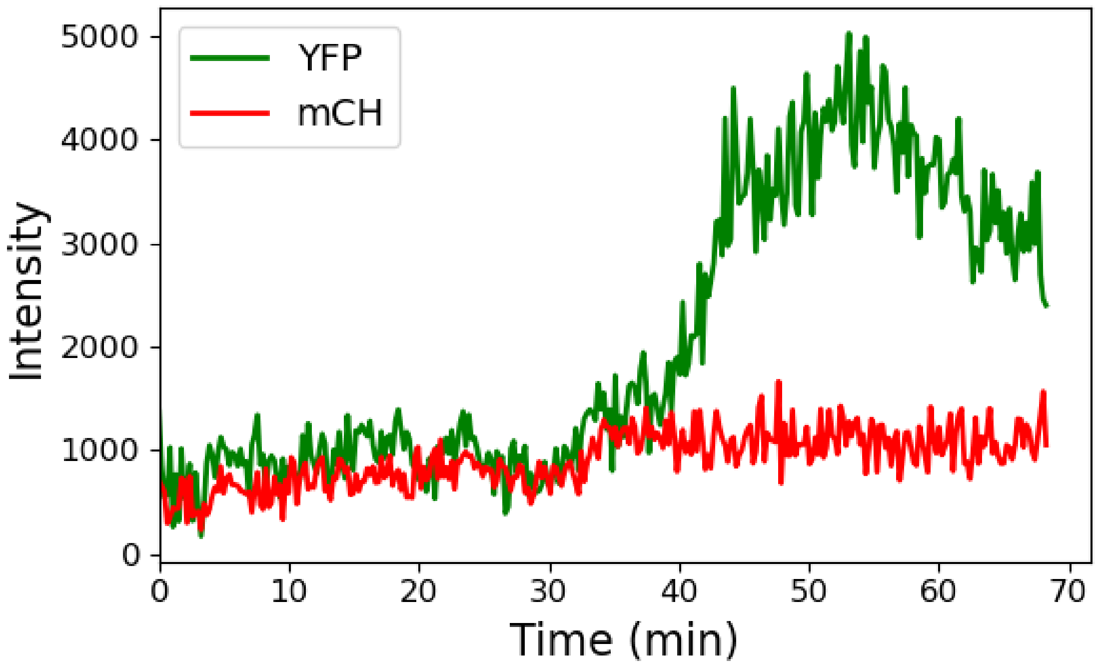
```python
# XY
x = df['x (um)'].values
y = df['y (um)'].values

points = np.array([x, y]).T.reshape(-1, 1, 2)
segments = np.concatenate([points[:-1], points[1:]], axis=1)

norm = plt.Normalize(0, len(x))
lc = LineCollection(segments, cmap='coolwarm', norm=norm)
lc.set_array(np.arange(len(x)))
lc.set_linewidth(2)

fig, ax = plt.subplots(figsize=(6, 6))
ax.add_collection(lc)
ax.set_xlim(x.min(), x.max())
ax.set_ylim(y.max(), y.min())  # invert Y-axis for top-left origin
ax.set_xlabel('x (um)')
ax.set_ylabel('y (um)')
ax.set_title('XY position')
ax.set_aspect('equal')

# Create a colorbar
divider = make_axes_locatable(ax)
cax = divider.append_axes("right", size="5%", pad=0.1)
cbar = plt.colorbar(lc, cax=cax)
```

```
cbar.set_ticks([])              # Remove numeric tick labels
cbar.set_label('start → end')

plt.tight_layout()

# Construct filename dynamically
base_name = os.path.splitext(os.path.basename(filepath1))[0]
save_path = os.path.join(filepath_out, f"{base_name}_trajectory.png")

plt.savefig(save_path, dpi=300)
plt.show()
```



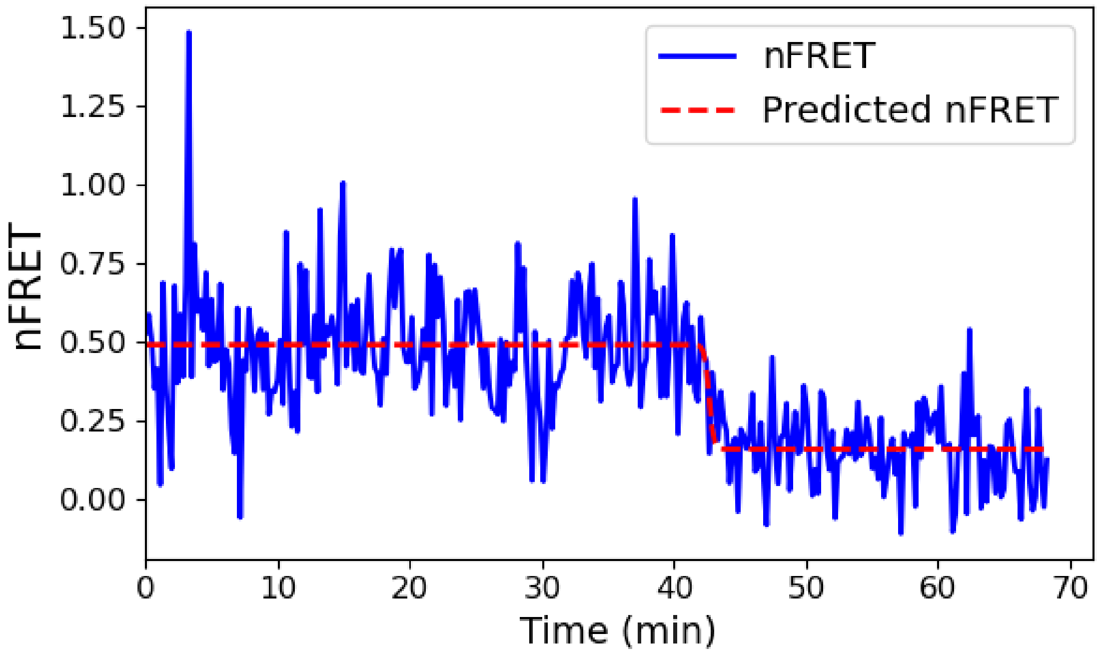

[271]:
```
# Intensity
color_YFP = 'green'
color_mCH = 'red'

plt.figure(figsize=(6,4))
plt.plot(df['Time (min)'], df['YFP'], label='YFP', color=color_YFP, linewidth=2)
plt.plot(df['Time (min)'], df['mCH'], label='mCH', color=color_mCH, linewidth=2)

plt.xlabel('Time (min)', fontsize=16)       # X-axis label size
plt.ylabel('Intensity', fontsize=16)  # Y-axis label size
plt.title(' ', fontsize=16)       # Title size
plt.legend(fontsize=14)                      # Legend text size
plt.tick_params(axis='both', which='major', labelsize=12)  # Tick labels size

plt.xlim(left=0)
plt.grid(False)
```

```
plt.tight_layout()
save_path = os.path.join(filepath_out, f"{base_name}_intensity.png")
plt.savefig(save_path, dpi=300)
plt.show()
```

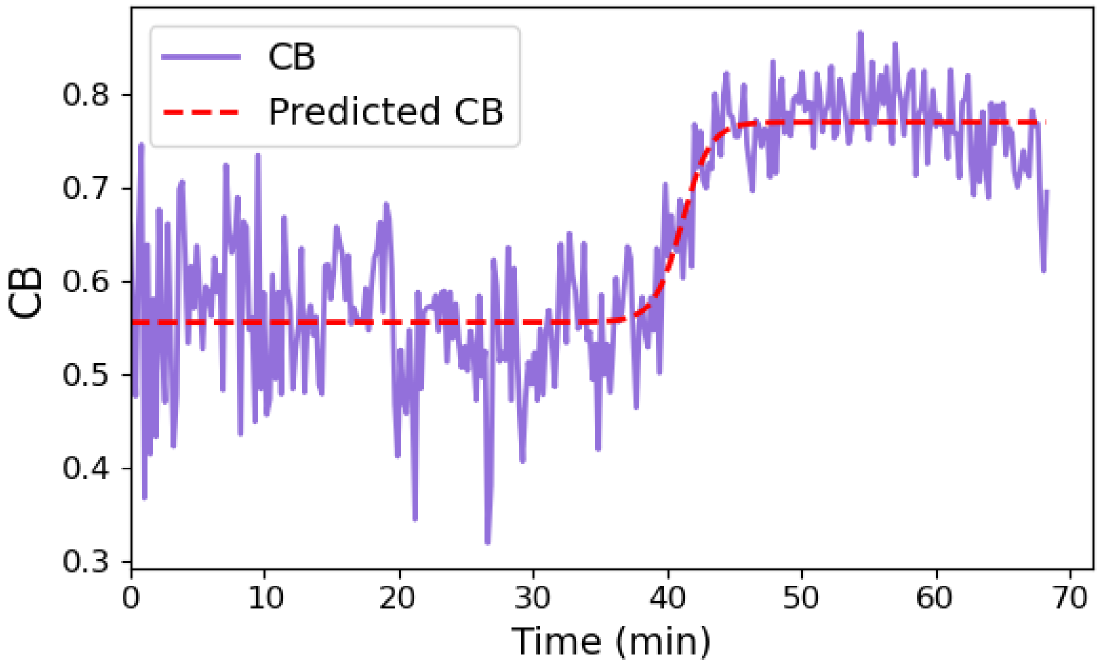



[272]:
```
# Sigmoid fit of nFRET
plt.figure(figsize=(6,4))
plt.plot(df['Time (min)'], df['nFRET'], label='nFRET', color='blue',␣
 ↪linewidth=2)
plt.plot(df['Time (min)'], df['predicted nFRET'], label='Predicted nFRET',␣
 ↪color='red', linewidth=2, linestyle='--')

plt.xlabel('Time (min)', fontsize=14)
plt.ylabel('nFRET', fontsize=16)
plt.title(' ', fontsize=16)
plt.legend(fontsize=14)
plt.tick_params(axis='both', which='major', labelsize=12)

plt.xlim(left=0) # can redo to left=100 to start at min=100, or xlim(10,20) to␣
 ↪only plot 10 to 20 min
plt.grid(False)
plt.tight_layout()
save_path = os.path.join(filepath_out, f"{base_name}_nFRET_Fit.png")
```

```
plt.savefig(save_path, dpi=300)
plt.show()
```




[273]:
```
# Sigmoid fit of CB
plt.figure(figsize=(6,4))
plt.plot(df['Time (min)'], df['CB'], label='CB', color='mediumpurple',␣
 ↪linewidth=2)
plt.plot(df['Time (min)'], df['predicted CB'], label='Predicted CB',␣
 ↪color='red', linewidth=2, linestyle='--')

plt.xlabel('Time (min)', fontsize=14)
plt.ylabel('CB', fontsize=16)
plt.title(' ', fontsize=16)
plt.legend(fontsize=14)
plt.tick_params(axis='both', which='major', labelsize=12)

plt.xlim(left=0) # can redo to left=100 to start at min=100, or xlim(10,20) to␣
 ↪only plot 10 to 20 min
plt.grid(False)
plt.tight_layout()
save_path = os.path.join(filepath_out, f"{base_name}_CB_Fit.png")
plt.savefig(save_path, dpi=300)
plt.show()
```


CB
Predicted CB
0.8
0.7
0.6
0.5
0.4
0.3
CB
0
10
20
30
40
50
60
70
Time (min)

```
[ ]:
```